AN ABSTRACT OF THE THESIS OF

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Title: ENZYMATIC FORMATION AND INFLUENCE OF VARIETY, MATURITY AND PROCESSING ON VOLATILES OF THE CARROT, DAUCUS CAROTA L.

Abstract approved: [redacted]
Dr. R. E. Wrolstad

Carrot volatiles were subjected to qualitative and quantitative analysis in a study of carrot flavor (aroma). Enzymatic formation of carrot volatiles and the influence of variety, maturity and processing on carrot volatile composition were investigated using a rapid technique employing gas-entrainment, on-column trapping of headspace volatiles present in aqueous carrot extracts. A suitable method for removing water from entrained volatiles was developed and the compounds analyzed using gas-liquid chromatography coupled with mass spectrometry (GLC-MS). Compounds were identified by comparing their mass spectra with reference spectra. GLC relative retention times were used to confirm the mass spectral identifications.

Twenty-three compounds were positively identified and seven compounds were tentatively identified in raw carrots. Those

compounds positively identified include diethyl ether, acetaldehyde, acetone, propanal, methanol, ethanol, α-pinene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, limonene, γ-terpinene, p-cymene, terpinolene, octanal, bornyl acetate, caryophyllene, terpinene-4-ol, β-bisabolene, γ-bisabolene, and carotol. (Underlined compounds had previously not been identified in the raw carrot.) Compounds tentatively identified include myristicin, 2-decenal, four sesquiterpenes and an aromatic compound with a M.W. of 134. No single compound was found that could be considered solely responsible for carrot aroma, however, acetaldehyde, sabinene, myrcene, terpinolene, and to a lesser extent caryophyllene and carotol, were considered important character-impact compounds in raw carrot aroma.

Experiments were conducted which demonstrated a characteristic raw carrot aroma could be regenerated from essentially odorless carrot substrates ("precursor" material) when the substrates were reacted with "flavorese" (flavor forming) enzymes prepared from raw carrots. "Flavorese" enzyme activity was erratic; raw carrot aroma not always being regenerated with enzyme treatment. Examination of headspace volatiles present in enzyme reaction mixtures produced no reproducible evidence for the enzymatic formation of compounds coinciding with the enzymatic formation of raw carrot aroma.

It is concluded that raw carrot aroma may result from a

complex interaction of several compounds or may be due to compound(s), possibly transient, unstable compounds, beyond the limits of detection of the analytical method used.

As well as qualitative, quantitative analysis was used for examining the influence of variety, maturity and processing on the volatile composition of carrots. Varieties studied included Imperator (Long Imperator Crookham), Nantes, Royal Chantenay, Autumn King, Oregon 4362 and Wisconsin 5. Considerable inherent variation in the concentration of volatiles was apparent, both within and between varieties. Varietal and maturity differences were quantitative rather than qualitative. The varietal variation in total essential oil content ranged from 5 ppm to 27 ppm, essential oil content increasing with maturity (in late season carrots).

Accumulation of ethanol, and to a lesser extent acetaldehyde, in late season carrots and in carrots under storage conditions, indicated anaerobic metabolism in "aging" carrot tissue approaching senescence.

Differences between canned, freeze dried and raw carrots were also mainly quantitative, major exceptions being the formation of ethane thiol, dimethyl sulfide and dimethyl substituted styrene compounds with canning. Canning resulted in an approximate 50% loss of "higher boiling" compounds, however, it also produced an increase in the "lower boiling" compounds (acetaldehyde, propanal, acetone,

methanol, ethanol's concentration was relatively unaffected), octanal, and 2-decenal. The concentration of methanol increased dramatically from approximately 0.05 ppm to approximately 60 ppm. Freeze drying resulted in an approximate 75% loss of total volatile content. Ethane thiol, dimethyl sulfide, acetaldehyde, octanal, 2-decenal and possibly dimethyl substituted styrene compounds, are considered important in canned carrot flavor (aroma).

Enzymatic Formation and Influence of Variety, Maturity and Processing on Volatiles of the Carrot, Daucus carota L.

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ENZYMATIC FORMATION AND INFLUENCE OF VARIETY, MATURITY AND PROCESSING ON VOLATILES OF THE CARROT, DAUCUS CAROTA L.

INTRODUCTION

Until recently the flavor chemistry of most vegetables, including the carrot, has been essentially ignored. In particular there have been very few reports on the enzymatic formation of flavors and on the influence of variety, maturity and processing on the volatile composition of vegetables. A knowledge of the biosynthesis of a flavor can provide the possibilities of treating a product before, during or after processing in order to retain or decrease flavor, or compensate for losses due to processing. A greater understanding of how volatile composition can be affected by variables such as variety, maturity and processing is of value to both the plant geneticist and the food processor. Varietal differences can be exploited by the plant geneticist, who by systematic breeding, can develop varieties with more desirable flavors for both the fresh market and for processing. A knowledge of processing effects and the influence of variety and maturity on volatile composition can permit the processor to make the best choice of variables such as time of harvest and processing conditions.

When this research was initiated there had been no basic studies reported on the volatile composition of the carrot root.

During the course of this study, Buttery et al. (1968) published a report characterizing several volatile components present in carrots. Quantitative information on the concentration of the volatiles present in raw carrots is incomplete however, making it difficult to estimate the significance of these compounds. As key compound(s) responsible for carrot aroma remain elusive, one objective of this study was to determine the identity and concentration of volatiles present and enzymatically induced in carrots in an attempt to elucidate which compounds are responsible for raw carrot flavor (aroma). Additional objectives were the determination of the influence of variables such as variety, maturity and processing on the volatile composition of carrots.

REVIEW OF LITERATURE

Gross Chemical Composition of Carrots

A knowledge of gross composition can be instructive in understanding flavor changes and development in a natural product. The gross composition of the edible part of the carrot (the root) is as follows (Watt and Merrill, 1963).

	Percent by Weight		
	Raw	Canned	Dehydrated
Water	88.2	92	4
Protein	1.1	0.6	6.6
Fat	0.2	0.2	1.3
Carbohydrate			
Total	9.7	6.5	81.1
Fiber	1.0	0.6	9.3
Ash	0.8	0.9	7.0
Ca	0.37	0.25	2.56
P	0.36	0.2	2.34
Fe	0.7	0.7	6.0
Na	0.47	2.36	2.68

Although the carrot has a chemical composition typical of many vegetables in most respects, the protein content is rather low and the sugar and mineral content fairly high.

The Volatile Composition of Carrots

Essential Oil of Carrot Seeds

The volatile composition of carrot seed oil has been thoroughly

investigated, mainly because of its importance as a raw material in the perfume industry.

The most recent study on the identification of volatile components in carrot seed oil was reported while this study was in progress by Seifert, Buttery and Ling (1968). These authors identified the following 23 components; the underlined compounds had not been reported previously: α-pinene, camphene, β-pinene, sabinene, myrcene, α-terpinene, p-cymene, limonene, γ-terpinene, linalool, terpinene-4-ol, α-terpineol, bornyl acetate, geraniol, geranyl acetate, caryophyllene, γ-decanolactone, β-selinene, coumarin, carotol, daucol, α-gurjunene and β-bisabolene. The presence of several unidentified compounds was also reported. The same authors reviewed the great many earlier reports listing identified and incompletely characterized compounds that had been found in carrot seed oil.

The composition of the oil varies considerably with variety and growing region, not only qualitatively but also quantitatively (Pigulevskii and Koraleva, 1961; Sorm and Urbank, 1948). For example the three main constituents in cultivated European and Japanese carrots are carotol (9-63%), geranyl acetate (0-48%), and epoxy dihydrocaryophyllene (2.5-20%) (Stahl, 1964). Undoubtedly differences in the reported compositions also reflect changes occurring in the oil due to different methods of isolation and identification.

Volatile Composition of the Carrot Root

It is relevant to consider why comparatively few studies have been made on the flavor of vegetables compared to fruits and essential oils. In a recent review on vegetable flavor components Bernhard (1966) discusses the difficulties of working with vegetable flavors. The flavors of many vegetables are difficult to describe and even worse to differentiate from each other. The flavor volatiles frequently occur in concentrations of only a few parts per million (ppm) surrounded by large amounts of other organic materials and huge quantities of water. Vegetables which have been investigated tend to be those with strong characteristic aromas such as onions, cabbage, turnips and rutabaga (Bernhard, 1966).

The earliest recorded attempt at examining carrot volatiles was reported by Konig and Kracht (1929) who found the presence of sulfur compounds in cooked carrots. Buttery and Teranishi (1961) demonstrated volatiles could be recovered from the headspace above cooked and frozen stored carrots, as well as other fruits and vegetables. These authors used a direct injection of aqueous vapors for gas-liquid chromatographic analysis. Several peaks were resolved but no compounds were identified. In 1968, Buttery et al. published a paper characterizing some volatile constituents in carrots (Imperator variety). Steam distillation with a Likens extraction head was used for preparing a carrot oil. The carrot oil, steam distilled at

atmospheric pressure, smelled like cooked carrots. Hydrocarbon and oxygenated fractions were separated by selective absorption on silica gel and the fractions examined by gas-liquid chromatography-mass spectrometric analysis. The hydrocarbons identified were: α-pinene, camphene, β-pinene, myrcene, α-terpinene, p-cymene, limonene, γ-terpinene, terpinolene, caryophyllene, β-bisabolene and γ-bisabolene. Oxygenated components identified were: heptanal, octanal, nonanal, 2-nonenal, terpinene-4-ol, α-terpineol, 2-decenal, bornyl acetate, 2,4-decadienal, biphenyl dodecanal, myristicin, and carotol. Several other compounds, mainly sesquiterpenes and aromatics, were incompletely characterized. Odor thresholds of a number of these compounds were measured in water solution and their total odor intensity contribution estimated. However, key compounds responsible for the characteristic raw and cooked carrot aroma were not elucidated by this study.

Enzymatic Development of Odor

Flavor perception is a complex sensation consisting primarily of odor and taste. Whereas taste is a relatively simple and limited response (receptors are only capable of distinguishing four basic tastes: sweet, bitter, salty and acid) odor perception is much more complicated there being about 2000 odors recognizable on a memory basis (Konigsbacher and Hewitt, 1964). The characteristic flavor of

fruits and vegetables and other fresh biological materials is due to compounds produced by metabolic processes which are believed to be mainly enzyme catalyzed. There is an abundance of evidence that many fresh flavors arise as a consequence of the interaction of enzyme(s) and substrate(s) (Flavor precursors) when the cellular integrity of tissues is destroyed (Schwimmer and Weston, 1961; Schwimmer, 1963; Konigsbacher and Hewitt, 1964; Yu, Olson and Salunkhe, 1968a, 1968b; and Fleming et al., 1968).

Enzymatic Regeneration of Flavor--the "Flavorese" Concept

For several years food scientists have been interested in the possibility of improving the flavor of processed food products by treatment with enzymes capable of regenerating volatile flavor compounds lost during processing. Food processing, particularly processing involving heating, can result in loss or change in the flavor of many fruits and vegetables. The role of enzymes in the regeneration of fresh flavor in processed foods is embodied in the "flavorese" concept of Hewitt et al. (1956) outlined in Figure 1.

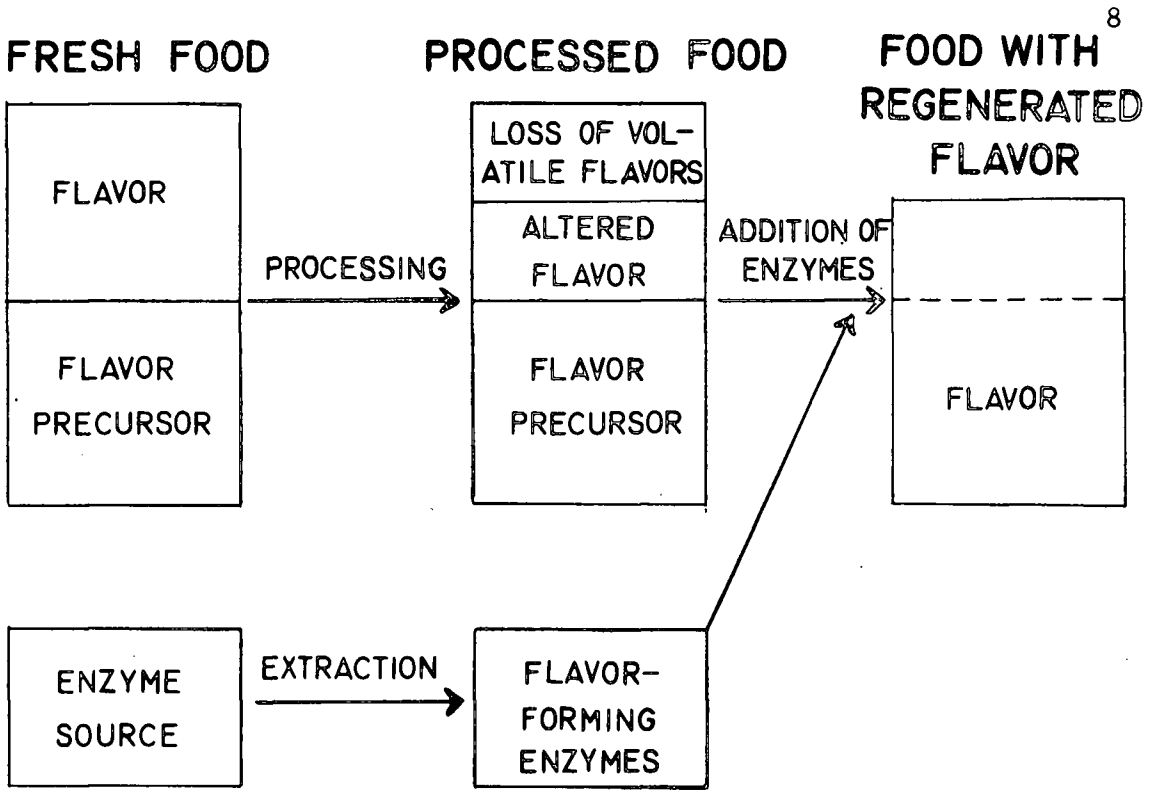

Figure 1. Enzymatic regeneration of flavor in processed food.

This scheme advanced the concept that whereas heat processing destroys or inactivates "flavorese" enzymes (enzymes that synthesize flavor) the precursors are sufficiently non-volatile and sufficiently stable to survive processing. On addition of the appropriate "flavorese" enzyme prepared from the fresh product, non-volatile precursor(s) are converted to volatile flavor components. This concept has been confirmed by several workers (Schwimmer, 1963) and has been reviewed by Hewitt (1963), Konigsbacher and Hewitt (1964), and Reed (1966). Only with a few vegetables which have very strong characteristic aromas has the nature of the substrate (precursor), flavor and enzymes been fairly well established. The Crucifera family (cabbage, mustard, horseradish and watercress) have been

studied extensively. Mustard oils (isothiocyanates) which are readily detected are synthesized from thioglycosides by the action of thioglycosidases (Reed, 1968). The flavor precursors of onion aroma have been found to be (among others) S-substituted cysteine compounds which are decomposed by sulfoxidase enzymes to produce sulfenic acids. The sulfenic acids further react to give sulfur compounds which are responsible for the biting, lachrymatory effect of onion aroma (Schwimmer, 1968). Recently Yu, Olson and Salunkhe (1968a, 1968b) have indicated amino acids can be converted into carbonyl compounds by crude enzyme extracts prepared from fresh tomatoes. However, no correlation was made between compounds enzymatically formed and the characteristic fresh tomato aroma. Konigsbacher and Hewitt (1964) claim the enzymatic enhancement of processed green bean aroma may be due to a small increase in the concentration of 2-trans hexenal. Similarly, Attaway and Metcalf (1966) indicate a slight enzymatic improvement in orange juice aroma may be due to a small increase in the concentration of limonene. In both of these instances the "enzymes" were not reported to be producing new compounds but were increasing the concentration of existing compounds.

In most studies concerning the enzymatic formation of aroma it has merely been established, using organoleptic evaluation, that a crude enzyme extract from a fresh product can regenerate the flavor of the processed product (Schwimmer, 1963; Reed, 1968). In

such cases the process is believed to be enzymatic as the "enzyme extracts" are heat labile, heat inactivated preparations failing to regenerate the fresh flavor. In some instances variations in and loss of enzyme activity have been reported. Miller (1957) reported erratic enzyme activity in the enzymatic regeneration of pea and bean aroma. No correlation was found between methods of enzyme preparation and enzyme activity. Repeated tests with the same enzyme preparations gave good activity (evaluated organoleptically) on one occasion and little or none on others. Variation in enzyme activity with crude enzyme preparations from tomatoes (Yu, Olson and Salunkhe, 1968b) and oranges (Attaway and Metcalf, 1966) has also been reported. In an interesting experiment described by Konigsbacher and Hewitt (1964) scientists in the French perfume industry used enzyme extracts to develop orange blossom aroma from an odorless orange blossom precursor preparation. Enzyme preparations displayed erratic activity and only produced the orange blossom aroma at a pH of 4.

The complexity of the situation and the specificity of "flavorese" enzyme preparations is demonstrated by the fact that apart from the enzyme systems described for the cabbage and onion families, commercially available enzymes have not been found which have the same effects as the "flavorese" enzyme preparations (Hewitt, 1963; Konigsbacher and Hewitt, 1964).

In summary, although the flavorese concept has potential

commercial application:

> The entire field of flavor enhancement or flavor production by enzymes is in its infancy. Therefore, most of the work has been done with foods in which a very potent, readily recognizable flavor could be produced . . . subtler changes in flavor which may be produced by enzyme action certainly deserve attention by enzyme chemists and food technologists (Reed, 1966).

Influence of Maturity, Variety and Processing on Carrot Volatile Constituents

Apart from the investigations on carrot seed oil there have been no studies reported on the influence of maturity and variety on carrot volatile constituents. Indeed, the influence of maturity and variety on the flavor chemistry of fruits and in particular vegetables has received little attention from horticulturalists or flavor chemists. However, recently there has been increased interest in the improvement of flavor in vegetable products by studies of flavor components. Johnson et al. (1968) reported variations of several ppm with variety and harvest in the concentrations of iso-amyl alcohol, pentanol and 3-hexen-1-ol in tomatoes. For instance, iso-amyl alcohol concentration varied from 1.8 to 13 ppm among varieties and the total quantity of the three volatiles varied from 7.4 to 40.2 ppm. Nelson and Hoff (1969) also reported small varietal variations in the concentration of acetaldehyde, acetone, methanol, ethanol, isovaleraldehyde and hexanal in tomatoes. Ethanol demonstrated the largest variation,

ranging from 14 to 62 ppm. Stevens (1970) has shown that quantitative differences in the concentration of 2-isobutyl thiazole, methyl salicylate and eugenol between tomato varieties is inheritable. Stevens (1967) and Stevens et al. (1967), demonstrated that quantitative differences in oct-1-en-3-ol and linalool among snap bean varieties is also inheritable. Intravarietal variation in the concentration of terpenoid compounds (terpinolene, 3-carene, γ-terpinene, terpinene-4-ol, citroneyllacetate and caryophyllene) has been demonstrated in black currants by Anderson and von Sydow (1964). Quantitative differences in some of these compounds existed among all six varieties studied. Attaway, Perringer and Barabas (1967) studied the influence of cultivars and maturity (season) on the terpenes present in the peel and leaf oils of "Dancy" tangerines, "Hamlin" oranges and "Marsh" grapefruit. The peel oils showed a large decrease in the percentage of oxygenated components, particularly linalool, during the course of one season. This was accompanied by a corresponding increase in the percentage of terpene hydrocarbons, particularly limonene. The total percentage of terpenes other than limonene held constant or decreased through the season. Consistent trends were not so evident in the leaf oils, however the total terpene percentage increased with maturity (at the end of the season).

No studies have been reported on the effects of canning on the volatile composition of carrots. However, in an ambitious undertaking

Self, Casey and Swain (1963) made a survey of the low boiling volatiles present in 13 cooked vegetables, including carrots, and also in beef, coconut, coffee and tea. After boiling for 30 minutes, carrots were found to contain methanal, methanethiol, propanal and/or acetone (not separated by conditions of analysis), ethanethiol, dimethyl sulfide, 2-methyl propanal and methanol. The concentration of compounds present was not determined. There have been very few reports on the effects of canning on the volatile composition of vegetables in general. Stevens et al. (1967) characterized 40 of the compounds present in canned snap bean liquor. However, no comparison was made with the fresh bean. Nelson and Hoff (1969) studied the effect of canning and storage on some volatiles present in three tomato varieties. Qualitative and quantitative differences were noted between the raw and canned samples. Dimethyl sulfide was formed by the heat processing and the concentrations of acetaldehyde, acetone, methanol and hexenal were altered by canning. Whereas there have been no basic studies reported on the effects of freeze drying on the volatile composition of carrots or any vegetable, the development of an off-odor which was reminiscent of violets and which coincided with a loss in color, has been reported by Ayers et al. (1964) for accelerated freeze dried diced carrots stored in the presence of oxygen. Systematic analysis revealed that the compounds mainly responsible for the off-odor were α and β-ionones, and β-ionone-5,6-epoxide. A quantitative

relationship was demonstrated between the loss of β-carotene (and its congeners) and the formation of the oxidation products, α-ionone, β-ionone and β-ionone 5,6-epoxide.

EXPERIMENTAL

Samples

The carrots (Daucus carota L.) used in this study were obtained from the Horticulture Department, Oregon State University. The carrots were planted on June 10, 1969, in a sandy-loam (Chehalis) soil. Varieties and breeding lines investigated in this study included Imperator (Long Imperator Crookham), Nantes, Royal Chantenay, Autumn King, Oregon 4362 and Wisconsin 5.

Sample Preparation

Canning of Carrots

Carrots, evenly sized to approximately 1-1/2 inches in diameter, were steam blanched for three minutes at 210°F before peeling with a Blakeslee abrasive peeler and slicing (1/4 inch slices) with a Hobart unit. The slices were canned in 303 plain body cans with C-enamel ends by heating for 30 min at 240°F in a still retort. Canned carrots were stored at 24°C.

Freeze Drying of Carrots

Carrots were diced with a Hobart unit to give 1/2 x 3/16 inch dices which were individually quick frozen at a temperature of -30°C

in a blast freezer. Frozen dices were accelerated freeze dried in a Hull freeze dryer (Model 651M - 9 WDF20) at a vacuum of 100 microns and a temperature programmed from 230-130°F for 15 hours. The dried product weighed 10% of the fresh weight. Freeze dried dices were stored under nitrogen in tightly capped jars at -30°C.

Preparation of Aqueous Carrot Extracts for Headspace Analysis of Volatile Constituents

During the course of this study aqueous carrot extracts were prepared from raw, canned and freeze dried carrots for headspace analysis of the volatile constituents.

Raw carrot extracts (pH 6.5) were prepared from washed, peeled and diced (1/4 inch dices) carrots by blending for 30 seconds in a Waring blendor in the proportions of 200 g carrots/200 ml distilled water. This homogenate was squeezed through four layers of cheesecloth to give a final extract volume of 250 ml. A 125 ml aliquot of the extract was immediately analyzed. In an effort to obtain representative sampling of carrot material the 200 g of carrot dices were randomly selected from approximately 700 g of diced carrots.

Canned carrot slices were drained, blotted and extracted in the same manner as the raw carrots except 200 ml of canned carrot liquor was used for the extraction instead of distilled water. This permitted a quantitative comparison on a fresh weight basis of the

volatile content of canned and raw carrots.

Freeze dried carrots were also reconstituted so as to produce carrots equal on a fresh weight basis to the unprocessed vegetable. Freeze dried carrots were reconstituted in a sealed flask (20 min at 25°C or 20 min at 50°C) in the proportions of 20 g carrots/180 g water. Reconstituted carrots were then blended for 30 seconds in the proportions of 200 g carrot/200 ml water; then filtered and analyzed in the same manner as described for raw and canned carrots.[1]

Enzymatic Regeneration of Carrot Aroma (Volatiles)

Under suitable reaction conditions the "flavorese" concept of Hewitt et al. (1956) was confirmed using carrots as a source of enzymes and precursor material. Several methods for preparing active "flavorese enzymes" and "precursor" material were attempted.

Preparation of "Flavorese" Enzymes

All operations were carried out in a 1°C room. Carrots were washed, peeled, rinsed in distilled water and diced before use.

Preparation of Acetone Powder (A). The method employed was similar to that of Schwimmer (1963). With equipment pre-cooled at -20°C diced carrots were homogenized for one min in chilled distilled

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[1] Refer to analysis of headspace volatiles.

water in a Waring blendor in the proportions of 100 g carrots/100 ml water. The homogenate was squeezed through four layers of cheesecloth and the filtrate centrifuged at 5000 g x 10 min. The protein fraction was precipitated from the resulting supernatant by the addition of acetone at -30°C. Acetone was added in the proportions of five volumes of acetone/one volume of supernatant, with gentle stirring over a 10 min period. The suspension was filtered by suction in a Buchner funnel using white crepe filter paper (Van Waters and Rogers) to facilitate rapid filtration. The residue was washed several times in the funnel with 200 ml portions of cold acetone. The resulting powder was dried for a few min in the air to remove excess solvent and then left for 16 hours at 1°C under vacuum over P_2O_5 to remove remaining traces of moisture and solvents. The dried powder was stored at -30°C, under nitrogen in tightly capped jars. Yield ranged from 0.2-0.5% on a fresh weight basis.

Enzyme extracts for examining "flavorese" activity were prepared from the powders simply by dissolving (suspending) the desired amount of powder (50 to 200 mg) in 25 ml of distilled water or 0.1 M phosphate buffer, pH = 6.5, for 15 min at room temperature.

Preparation of Acetone Powder (B) by Direct Acetone Extraction. An acetone powder was prepared using conventional methods consisting of blending diced carrots for two min in a Waring blendor with five volumes of acetone chilled initially to -30°C. The residue

was filtered, washed, dried and stored in the same manner as previously described. The yield was approximately 6% on a fresh weight basis.

Enzyme extracts for examining "flavorese" activity were prepared by suspending the desired amount of powder in distilled water or phosphate buffer pH 6.5 (2-6 g/125 ml) and gently stirring for three hours at 1°C. The enzyme extract was prepared by filtering the suspension through a glass wool plug.

Preparation of Acetone Power (C) by Direct Extraction in the Presence of Polyethylene Glycol (PEG. This method is the same as the one used by Arakji (1968) for extracting pectic enzymes from cranberries. Diced carrots (100 g) were added to 100 ml of 2% PEG (M.W. 6000) dissolved in 0.1 M phosphate buffer, pH 6.5. Acetone at -30°C was added in the ratio of 1:5 (suspension to acetone). The mixture was blended, filtered, washed, dried and stored in the same manner as described previously. Yield was approximately 6% on a fresh weight basis. Enzyme extracts were prepared from this powder by using the same extraction technique described in the previous enzyme preparation.

Preparation of Acetone Powder (D) in the Presence of Polyvinyl Pyrrolidone (PVP). A modification of the procedure described by Loomis and Battaile (1966) was used. Diced carrots (200 g) were frozen in liquid nitrogen and blended for 30 seconds in a Waring

blendor. The liquid nitrogen frozen powder was suspended in 600 ml of 0.1 M phosphate buffer, pH 7.4, containing 200 g of PVP. This suspension was gently stirred for 30 min then squeezed through a nylon cloth. The residue was re-extracted with 400 ml of buffer and the combined filtrates centrifuged at 20,000 g x 15 min to remove particulate matter. From the clear supernatant the proteins were precipitated, filtered off, washed, dried and stored in the same manner described under preparation of acetone powder A. The yield was approximately 0.2% on a fresh weight basis. Enzyme extracts were prepared from this powder using the same method described under preparation of acetone powder (A).

Preparation of Substrate ("Precursor") Material

Preliminary investigations had indicated dehydrated carrots were an undesirable form of "precursor" owing to a strong, caramelized odor that tended to mask the more subtle raw carrot odor induced enzymatically. Therefore, improved "precursors" (substrates) with minimized background odor, were investigated.

Freeze Dried "Precursor". Reduced pressure steam distillation was used for preparing the main source of "precursor" material. This equipment (Figure 2) was also used for recovery of the volatile compounds present in raw carrots. Carrots were washed, peeled, sliced (1/4 inch slices) and blanched to negative peroxidase activity

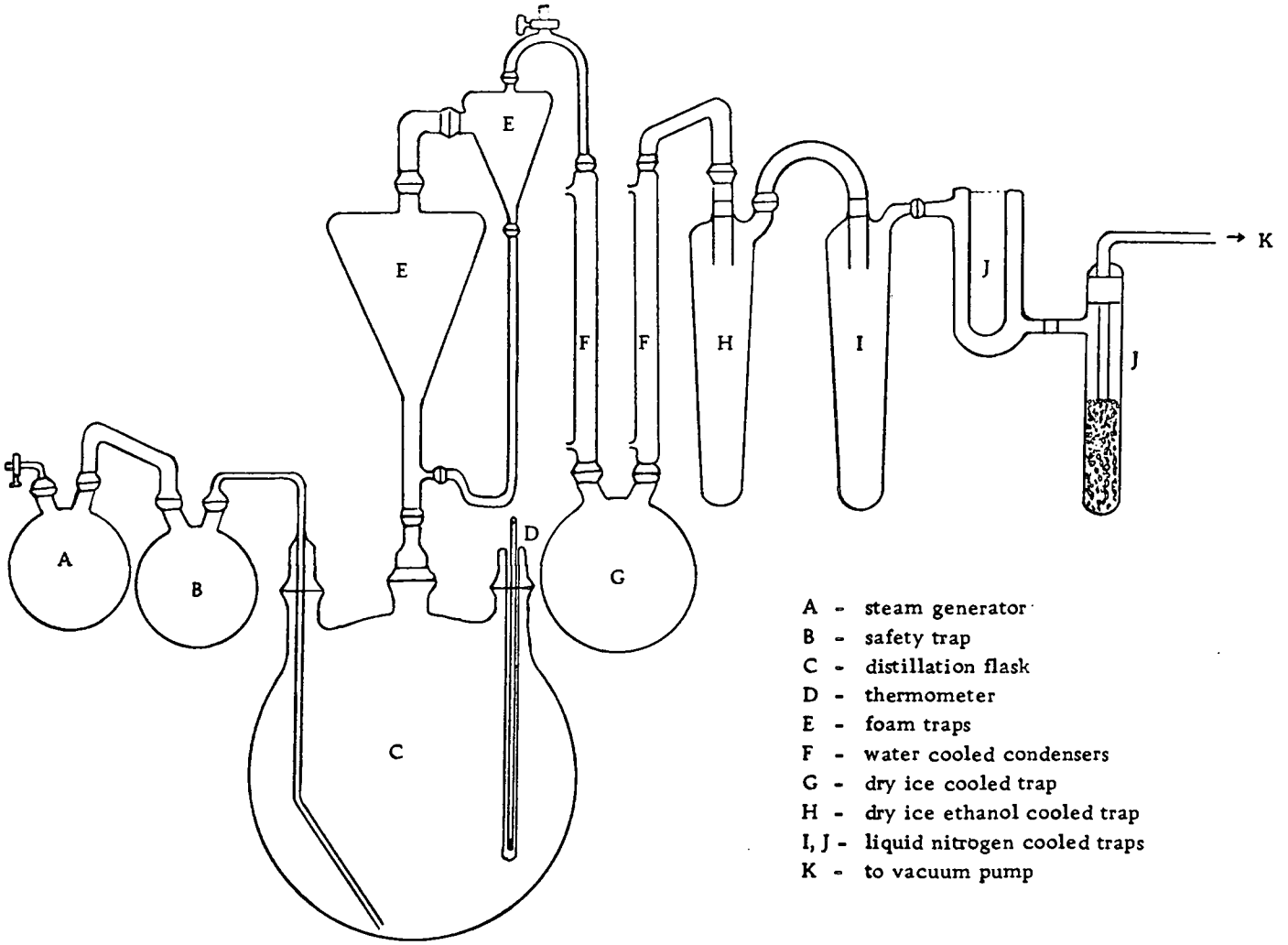

Figure 2. Vacuum steam distillation apparatus.

(three min at 210°F) in a steam box. A total of ten pounds of carrots were blended for one min in five liters of distilled water in a Waring blendor (in batches of two pounds carrots/one liter of water). The total homogenate was steam distilled at approximately 0.5 mm Hg for three or eight hours in the apparatus shown in Figure 2. The temperature of the carrot puree was maintained at 20-22°C during the distillation. The distillate containing carrot volatiles was condensed in a series of cold traps and extracted and concentrated for future analysis as described in the recovery of volatiles section in the Appendix. The residual homogenate was transferred to glass trays, frozen at -30°C and freeze dried in a Hull freeze dryer at a vacuum of 50 microns and a temperature programmed from 220-100°F for 16 hours followed by eight hours isothermally at 100°F. The dried product was powdered with a mortar and pestle and stored at -30°C under nitrogen in tightly capped jars. The yield was 10% on a fresh weight basis. On reconstitution this product was bland to the taste, essentially odorless and not recognizable as carrot.

Nitrogen Purged "Precursor". An alternative source of "precursor" was prepared as follows: 250 g of diced carrots were blanched in 600 ml of boiling distilled water for two min, cooled in an ice bath to 30°C, homogenized in a Waring blendor for 60 seconds and filtered through four layers of cheesecloth. The filtrate was gently stirred in a beaker on a combination hot plate-magnetic stirrer at a

temperature of 40°C for periods of up to three hours. Removal of volatiles was aided by entrainment in a stream of nitrogen which was bubbled through the extract at a rate of 400 ml/min. The final product, which was used for analysis immediately, was essentially odorless and not recognizable as carrot.

Nitrogen Purged Cooked "Precursor". Two hundred and fifty grams of diced carrots were boiled in 600 ml of water for 30 min and homogenized and nitrogen purged as previously described. The final product retained a weak background cooked carrot aroma.

Determination of "Flavorese" Activity

Under suitable reaction conditions "flavorese" enzyme preparation resulted in the regeneration of a characteristic raw carrot aroma when added to the essentially odorless substrate ("precursor") materials. Regenerated volatiles were subjected to both subjective (organoleptic) and objective (instrumental) analysis.

"Flavorese" Reaction Conditions. The reaction conditions used were similar to those reported by earlier workers (Schwimmer, 1963; Hewitt, 1963). For each test the following samples were prepared: (1) An enzyme and precursor sample; (2) A precursor only control sample; (3) An enzyme only control sample, and (4) A precursor and heat inactivated enzyme control sample. These samples were incubated in 250 ml glass stoppered conical flasks in a shaking

water bath at 37°C for the desired time (usually 90 min) before being analyzed. Detailed conditions used were:

(1) Enzyme and precursor sample. Precursor and enzyme were mixed in a final volume of 125 ml of distilled water or 0.1 M phosphate buffer, pH 6.5. Combinations examined included:

(a) 25 ml enzyme extracts of acetone powders A or acetone powders D added to 4 g of freeze dried precursor material which had been reconstituted to 100 ml at 37°C for 15 min.

(b) The desired quantity of acetone powders A or D (usually 50 mg) directly suspended in 125 ml of nitrogen purged "precursor" material.

(c) 4 g of freeze dried "precursor" directly reconstituted in 125 ml enzyme extracts from acetone powders B or C.

(2) Precursor only control sample. This control consisted of 4 g of freeze dried precursor reconstituted to a final volume of 125 ml in water or buffer; or 125 ml of nitrogen purged precursor material.

(3) Enzyme only control sample. Enzyme extracts were made up to a final volume of 125 ml in water or buffer.

(4) Precursor and heat inactivated enzyme control sample. Precursor material and enzyme extracts that had been heat inactivated

in a boiling water bath for seven min were made up to a final volume of 125 ml in water or buffer.

After incubation coded samples were submitted to sensory evaluation, initially on an informal basis with small groups of three to five trained personnel. Judges were asked if they could detect differences in aroma, to describe these differences and in particular to determine which samples had a characteristic raw carrot aroma. In some instances samples that appeared active were further compared with the various controls in triangular tests involving ten judges.

Analysis of Headspace Volatiles

The evaluation of enzyme activity by sensory methods is somewhat subjective. It was desirable to develop a suitable objective analytical method for determining small concentrations of volatiles released in an aqueous enzyme reaction mix of rather small volume. The conventional flavor chemistry techniques of distillation and/or solvent extraction followed by concentration steps were not suited for rapid, routine evaluation of the many enzyme reaction samples and controls. For similar reasons the conventional methods were also not suited for analyzing the large number of carrot samples investigated in subsequent studies on different carrot varieties and the influence of maturity and processing on carrot volatiles. A suitable technique

was developed by modifying the gas entrainment, on-column trapping technique developed by Morgan and Day (1965) and incorporating the modification described by Bills (1966). A diagram of the headspace assembly is shown in Figure 3. The 20 ml Kimble vial was replaced by a 250 ml screw cap reagent bottle. This allowed an extension of sample size from 10 ml to 125 ml which was well suited for the enzyme reaction mixture volumes (125 ml) and also permitted a more representative sampling of aqueous carrot extracts. The nitrogen inlet needle was extended to five inches in length and a magnetic stirring bar (1-1/2" x 1/4") included in the 250 ml bottle. The efficiency of volatile entrainment was improved by stirring and heating the sample in a water bath heated by a combination hot plate-magnetic stirrer as shown in Figure 3. A further modification was the inclusion of a precolumn containing a drying agent between the headspace entrainment ensemble and the gas chromatograph column. A nitrogen flow rate (entrainment rate) of 15 ml/min was used throughout this study.

In preliminary work carrot seed oil (Norda) was used in model system studies to determine the parameters which gave optimum conditions for recovery of volatiles from the headspace entrainment system. The carrot seed oil was chosen as it is a convenient standard source of the range of compounds found in carrots. One µl samples of carrot seed oil were thoroughly mixed in 125 ml of distilled water in 250 ml reagent bottles and the headspace analyses run. By

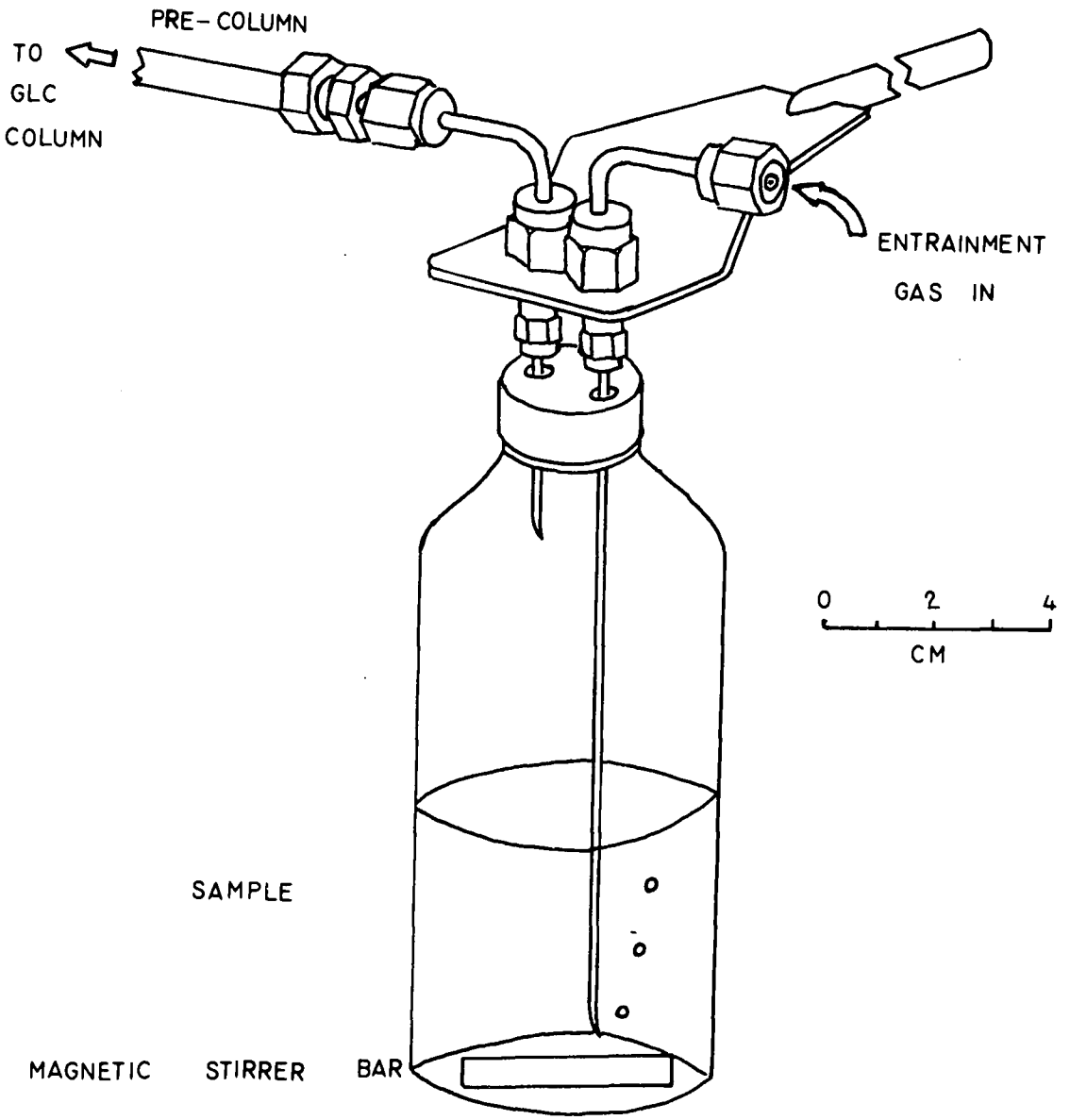

Figure 3. Headspace entrainment assembly used for the analysis of volatile constituents.

comparison with a direct on column injection of 1 μl of carrot seed oil the percentage recovery of a representative range of compounds under various conditions was determined. Parameters investigated included the influence of bath temperatures ranging from 25°C to 90°C and the effect of increasing entrainment times from five min to as long as 30 min. The GLC conditions used were as described in the gas liquid chromatography procedures section.

Gas Liquid Chromatography Procedures

In preliminary experiments, several 1/8" O.D. stainless steel packed columns were investigated to determine which liquid phases and what conditions gave the best separation of compounds in carrot seed oil and in aqueous carrot extracts.

Columns examined included: 7% Carbowax 20 M on 80/100 mesh Gas-Chromosorb Q, 10' x 1/8"; 5% SF96-50 containing 5% Igepal CO-880 on 80/100 mesh AW-DMCS Chromosorb G, 10' x 1/8"; 5% Apiezon L on 80/100 mesh AW-DMCS Chromosorb G, 10' x 1/8", 20% 1,2,3-tris (2 cyano ethoxy)-propane (TRIS) on 60/80 mesh Celite 545, 12' x 1/8", and 3% butanediol succinate (BDS) containing 0.5% igepal CO-880 on 100/120 mesh Chromosorb G. Unless otherwise stated an Aerograph 1520 equipped with a hydrogen flame ionization detector and connected to a Speedomax H recorder (1 mv, 1 second full scale response) was used. Various effluent splitters

ranging from 3:1 (air:detector) to 19:1 were used for evaluating GLC effluent odors during the course of the study. Carbowax 20 M gave the best overall separation of compounds and was used to the greatest extent. It was complemented well by SF96-50. The TRIS column gave the best separation of "lower boiling" compounds present in aqueous carrot extracts.

Gas Liquid Chromatography Combined with Rapid Scan Mass Spectrometry (GLC-MS)

GLC-MS was used to separate and identify a range of compounds present in the carrot seed oil. These identifications were useful in determining optimum conditions for recovery of the range of compounds present in raw carrots. The following operating conditions were used:

GLC

Instrument	F & M 810
Detector	Hydrogen flame
Detector temperature	225°C
Injection	On column, 0.5 µl carrot seed oil
Injector temperature	175°C
Column	Carbowax 20 M
Column temperature	isothermally at 75°C for 17 min then 4°/min to 195°C and held

Flow rate	25 ml/min of Helium
	M.S.
Filament current	20 eV source, 40 μA 70 eV source, 40 μA
Electron voltage	20 eV and 70 eV
Accelerating voltage	3000 V
Analyzer pressure	1×10^{-6} Torr
Multiplier voltage	1·60 KV
Scanning speed	2.5 seconds from m/e 25 to 250, and 5.0 seconds from m/e 25-250 for compounds with longer retention times

This instrument, an Atlas CH-4, is a Nier-type (nine inch, 60 degree sector) single-focusing mass spectrometer. Volatiles were separated by GLC prior to their entry into the dual ion source of the MS. The GLC was fitted with a 9:1 splitter so that 10% of the column effluent went to the hydrogen flame detector. The MS was equipped with an EC-1 intake valve which was adjusted to permit approximately 10% of the remaining column effluent to enter the ionization chamber, the remaining effluent being vented into the air through a heated tube, allowing the simultaneous evaluation of the odor of compounds being analyzed. The effluent was split in the ion source with 50% going to the 20 eV source and 50% going to the 70 eV source. As the 20 eV source operates at less than the ionization potential of the carrier

gas (He), but above that of organic compounds, it could be used as a chromatographic readout. The 70 eV source provides the ionization used to obtain mass spectra which were recorded on a Honeywell 1508 Visicorder.

Removal of Water Entrained During Headspace Analysis

Before attempting to use GLC-MS analysis for examining the enzyme reaction samples and controls, aqueous extracts of the carrot root were examined in an effort to identify the volatiles present in these extracts. It became apparent that a considerable quantity of water was being entrained with the volatiles by the headspace entrainment conditions that were necessary to recover higher boiling compounds from the aqueous extracts. Although this did not interfere with detection of compounds by flame ionization it did interfere with M.S. and thermal conductivity analysis. Large amounts of water also interfere with infrared analysis. Therefore, methods for the preferential removal of moisture from the entrained volatiles were investigated. Model system studies were used to determine which drying agents enabled the efficient selective removal of water from entrained volatiles. Drying agents were loosely packed between glass wool plugs in pyrex pre-columns inserted in the headspace entrainment system as shown in Figure 3. The 1/4" o.d. x 3" pyrex pre-columns were fitted with teflon ferruled swagelok fittings and

permitted packings of 50-1000 mg of desiccants as desired. A wide range of desiccants were examined, including anhydrous potassium carbonate, anhydrous calcium sulfate, anhydrous sodium sulfate, Sephadex (G-100), silica gel, and Linde molecular sieve 3A (the molecular sieve 1/16" pellets were tightly packed in a 10 ml volume pre-column). The removal of water by its conversion to acetylene and hydrogen by calcium carbide and calcium hydride in pre-columns was also investigated. In some instances the pre-columns were heated. A thermocouple was attached to the outside of the pre-columns and the tube wrapped with 1/2 inch wide heating tape connected to a variac for temperature control. The following conditions were used for the determination of water entrained by the headspace analysis procedure:

Sample size	125 ml of distilled water
Purge time and rate	15 min at 15 ml/min of nitrogen
Water bath temperature	$68^{\circ}C \pm 1.0^{\circ}C$
Instrument	Aerograph 700
Detector	Thermal conductivity
Detector temperature	$200^{\circ}C$
Filament current	150 ma
Column	Carbowax 20 M
Column temperature	Isothermal at $75^{\circ}C$ for 17 min; then 4°/min to $195^{\circ}C$
Flow rate	25 ml/min of nitrogen

As flame ionization detectors are insensitive to water, thermal conductivity was used to estimate the amount of moisture present in the entrained volatiles. A standard curve for peak height versus amount of water was prepared by direct injection of known amounts of water, and acetone solutions containing a known amount of water, as described by Francis Bennett (1964). The standard curve was linear for low quantities (up to 500 μg) however, owing to the tailing of the water peak larger quantities of water were estimated by comparing peak sizes with that obtained from injections of standard amounts of water. As some volatile compounds, particularly polar (oxygenated) compounds, were being retained to varying degrees by the desiccants the recovery of carrot seed oil compounds from the various desiccants was investigated using the following conditions:

Sample size	1 μl carrot seed oil in 125 ml of water
Purge time and rate	15 min at 15 ml/min, nitrogen
Water bath temperature	$68^{\circ}C \pm 1.0^{\circ}C$
Instrument	Aerograph 1520, hydrogen flame detector
Detector temperature	$225^{\circ}C$
Column	Carbowax 20 M
Column temperature	Isothermal at $75^{\circ}C$ for 17 min; then 4°/min up to $195^{\circ}C$ and held
Flow rate	25 ml/min of nitrogen

The solution arrived at which permitted GLC-MS analysis of the range of compounds present in aqueous carrot samples was a combination of using a pre-column containing anhydrous K_2CO_3 and the EC-1 valve on the mass spectrometer.[2]

GLC-MS Analysis of Aqueous Carrot Samples

GLC-MS was used for analyzing enzyme reaction mixtures, and raw, canned and freeze dried carrot extracts. The volatiles, for convenience, were divided into "lower boiling" and "higher boiling" compounds and analyzed under the following conditions.

Analysis of "Lower Boiling" Compounds

GLC Conditions

Sample size	125 ml
Purge time and rate	10 min at 15 ml/min Helium
Water bath temperature	$50^{\circ}C \pm 1.0^{\circ}C$
Instrument	F & M 810, hydrogen flame detector
Detector temperature	$225^{\circ}C$
Column	TRIS
Column temperature	Isothermal at $37^{\circ}C$
Flow rate	25 ml/min, Helium

[2] Refer to Results and Discussion.

Operating conditions for the mass spectrometer were the same as previously described.

Analysis of "Higher Boiling" Compounds

GLC Conditions

Sample size	125 ml
Purge time and rate	15 min at 15 ml/min Helium
Water bath temperature	$68^{\circ}C \pm 1^{\circ}C$
Instrument	F & M 810, hydrogen flame detector
Detector temperature	$225^{\circ}C$
Column	Carbowax 20 M or SF 96-50
Column temperature	Isothermal at $75^{\circ}C$ for 17 min then 4°/min to $195^{\circ}C$ and held
Flow rate	25 ml/min, Helium

Operating conditions for the mass spectrometer were the same as previously described.

Quantitation of Headspace Volatiles

Standard curves of peak area (determined by triangulation) versus concentration were prepared from known concentrations (in ppm) of authentic compounds recovered from aqueous extracts of reconstituted freeze dried "precursor" material. The precursor material was an ideal medium because of its low volatile background and its close simulation of the chemical nature of carrots. It was

possible to determine the peak areas obtained from a given concentration of several compounds in one analysis. Analysis conditions were identical to those already described for both "lower" and "higher" boiling compounds.

Internal standards were added to all samples analyzed for quantitative data by the headspace technique. This permitted correction for possible variations in sensitivity of the method. Heptanol was selected as the internal standard for use in determining "higher boiling" compounds as it had a retention time which differed from other compounds and it had a high enough boiling point and sufficient water solubility to ensure its recovery from aqueous extracts was not limiting. A 1 ml aliquot of 25 ppm solution of 1-heptanol was added to each 125 ml sample to be analyzed, resulting in a final concentration of 0.2 ppm. Methyl acetate at a final concentration of 0.2 ppm was selected as the internal standard used when examining the "lower boiling" compounds.

RESULTS AND DISCUSSION

Analysis of Headspace Volatiles

An analytical procedure was developed for isolating, concentrating and detecting the enzymatic formation of volatiles in aqueous carrot extracts, and for determining the influence of variety, maturity and processing on carrot volatiles. The system consisted of an on-column trapping technique in conjunction with GLC-MS analysis.

Preliminary experiments using carrot seed oil in model system studies determined optimum conditions for the recovery of a representative range of volatiles from the headspace system. A range of low to high boiling compounds were identified in the carrot seed oil, using GLC-MS (Table 1, Figure 4). The identifications confirmed the earlier work of Seifert, Buttery and Ling (1968) who reported these compounds and several others in carrot seed oil. Headspace analysis conditions examined included sample sizes from 10 to 125 ml, sample (bath) temperatures ranging from 25 to 80°C and nitrogen purge times ranging from 5 to 30 min. As entrainment of water which is proportional to bath temperature and purge time was a limiting factor,[3] the minimum conditions providing sufficient recovery of compounds for identification purposes were selected. These conditions were 15

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[3] Refer to removal of water from entrained volatiles.

Table 1. GLC-MS identification of compounds in carrot seed oil, using a Carbowax 20 M column. Gas chromatogram is shown in Figure 4.

Peak no.	Compound	tr/tr	M.S. identification	M.S. reference	GLC confirmation	Aroma
1	α-Pinene	0.46[a]	+	Ryhage & von Sydow	+	+
2	Camphene	0.57[a]	+	(1963)	+	
3	β-Pinene	0.69[a]	+	ibid	+	+
4	Sabinene	0.76[a]	+	ibid	+	+
5	Myrcene	0.81[a]	+	ibid	+	+
6	Limonene	1.00[a]	+	ibid	+	+
7	γ-Terpinene	1.21[a]	+	ibid	+	+
8	p-Cymene	1.38[a]	+	ibid	+	+
9	Caryophyllene	1.00[b]	+	ASTM (1969)	+	+
10	Linalool	1.04[b]	+	ibid	+	+
11	β-Bisabolene	1.14[b]	+	Libbey (1969), Buttery et al. (1968)	+	+
12	Carotol	1.42[b]	+	ibid	+[c]	+

[a] tr/tr relative to limonene

[b] tr/tr relative to caryophyllene

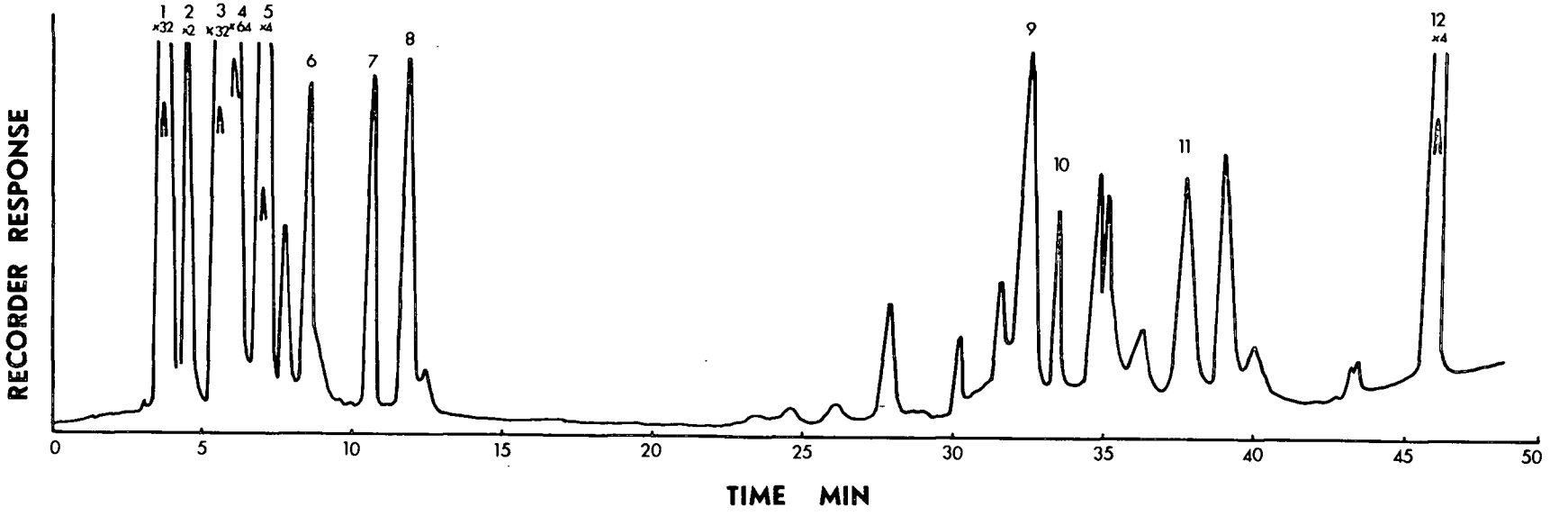

Figure 4. Analysis of carrot seed oil volatiles using a Carbowax 20 M column.

min at a nitrogen purge rate of 15 ml/min and a sample (bath) temperature of 68°C. Shorter purge times and/or lower bath temperatures did not recover surficient quantities of higher boiling compounds for identification purposes. For instance, 7.5 min at 68°C provided a sufficient recovery of monoterpene hydrocarbons but an insufficient recovery of the sesquiterpene hydrocarbons (e.g. caryophyllene, bisabolene) and the oxygenated terpenes linalool and carotol.[4] The recovery of these compounds was less than 15% the recovery of monoterpene hydrocarbons and was insufficient for MS analysis. In contrast, raising the bath temperature above 68°C increased the recovery of these higher boilers. However, it resulted in the entrainment of excess amounts of water. Under standard conditions (15 min at 68°C) the reproducibility of the recovery of compounds in carrot seed oil was ± 7% or better. There was no gas chromatographic evidence of isomerization or rearrangements of compounds under these conditions. Saturation of aqueous extracts with sodium sulfate as recommended by Morgan and Day (1965) was no advantage under the conditions used. Figure 5 illustrates the successful application of these conditions to the analysis of raw carrot extracts. Component identifications are listed in Tables 5 and 6. A wide range of compounds were recovered in substantial amounts, ranging from compounds such as acetaldehyde (M.W. of 44, b.p.

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[4]Terpene structures are listed in the Appendix.

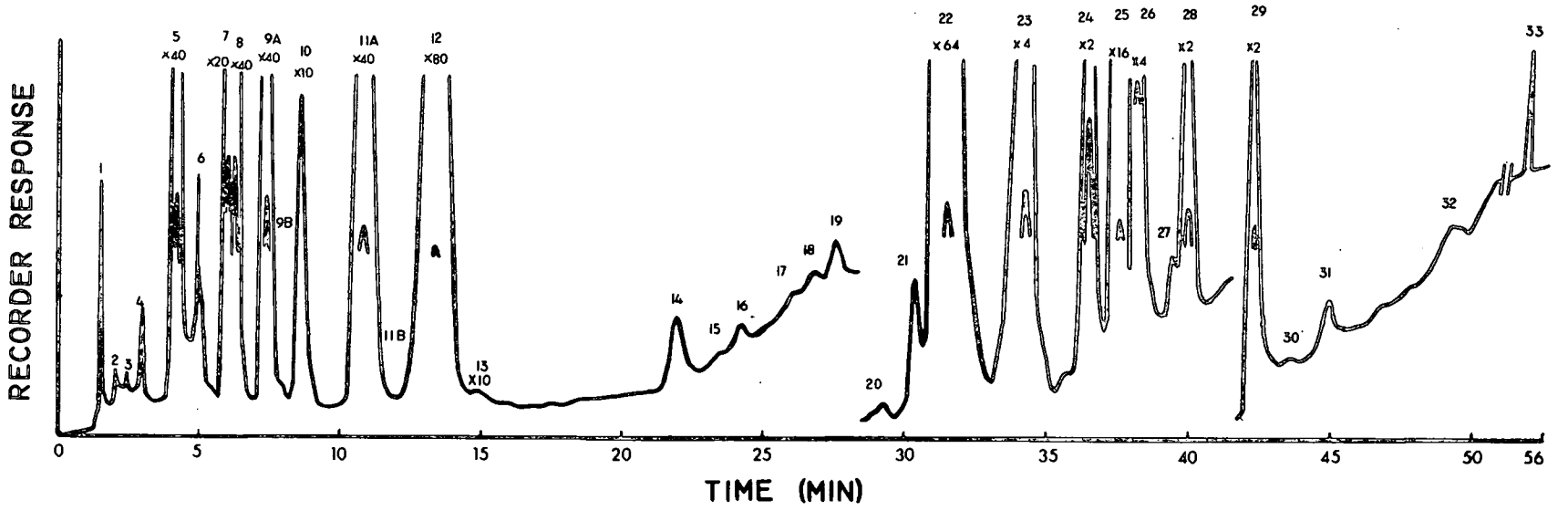

Figure 5. Analysis of the headspace volatiles present in an aqueous extract of raw carrots, using a Carbowax 20 M column.

^{760} = 21°C) to compounds such as caryophyllene (M.W. of 204, b.p. ^{14} = 130°C). The method is well suited for obtaining data from many samples as it is rapid, convenient and only requires a modest sample size. In contrast, conventional methods utilizing distillation and/or solvent extraction require large quantities, are time consuming and often use elevated temperatures for long periods of time. Conditions such as these can produce artifact formation. The evaporation (concentration) of large quantities of solvent can result in the loss of lower boiling compounds, solvent impurities and chemical changes. A drawback of most enrichment procedures such as the one used in this study is that the efficiency of enrichment is not the same for all compounds. For instance, higher boiling compounds (and more polar compounds) are less efficiently recovered than lower boiling compounds as was illustrated in the model system studies. However, in this study these differences are compensated for in the quantitative studies by preparing standard curves of peak area versus concentration of compound in ppm in solution.

Undoubtedly changes in volatiles due to enzymatic reactions occur as a result of tissue rupture during extraction. The headspace vapors in equilibrium above a carrot homogenate may differ to those above intact, raw carrots, however the blended form would more closely simulate the release of volatiles occurring when carrots are masticated. Even if enzymatic changes were occurring, the blended

aqueous carrot extracts retained a strong characteristic raw carrot aroma. A weaker raw carrot aroma was still present at the completion of headspace analysis.

Removal of Water From Entrained Volatiles

The purpose of this study was to selectively remove the water from entrained volatiles before entering the GLC column. An estimated 10 mg of water was entrained with volatiles under standard headspace analysis conditions. The water eluted as a large, tailing peak on all columns examined. On Carbowax 20 M water appeared in the region of sabinene, the peak tailing out past caryophyllene (Figure 5). Water not only shortens column life but also can interfere with thermal conductivity, M.S. and infrared analysis. In M.S. large quantities of any compound, including water, can overload the mass spectrometer resulting in an increase in sample pressure in the ion source. Under these conditions secondary reactions between ions and molecules can occur producing unreliable mass spectra (McLafferty, 1966). Also, background peaks may increase substantially as a result of sample displacement of background material from the inlet system walls. If the M.S. has only total ionization readout the large water peak can mask the presence of other peaks (Heins et al., 1966). A wide range of desiccants were examined for both removal of water and recovery of carrot seed oil compounds, using model system studies.

The choice of desiccant is critical in that it must remove water and yet remain inocuous to other compounds.

As illustrated in Table 2 several desiccants were effective at removing water when used at room temperature, however, the recovery of higher molecular weight compounds particularly polar (oxygenated) compounds from the desiccants, tended to be very low or zero. This would indicate a possible adsorption or condensation effect. Potassium carbonate was the most promising desiccant and Table 3 illustrates the effect of increasing pre-column temperatures on the efficiency of water removal and compound recovery using this drying agent. As the temperature increased the recovery of higher boiling compounds (caryophyllene, linalool, bisabolene, carotol) increased but the water removing efficiency decreased. As condensation is less likely at the higher temperatures the results suggest an adsorption effect which is proportional to molecular weight and polarity. As would be expected, oxygenated compounds such as linalool and carotol are adsorbed or "bound" more strongly than hydrocarbons. These compounds behaved in sympathy with the water; increased retention of water coinciding with increased retention of compounds.

A wide range of conditions including various quantities of desiccants (ranging from the minimum amount necessary on a molar reaction basis to several fold this amount) and a wide range of pre-column temperatures were investigated in an attempt to circumvent this

Table 2. Percent recovery of carrot seed oil volatiles from and percent removal of water by various desiccants at 25°C.

	Dessicant						
Compound	K_2CO_3 (300 mg)	$CaSO_4$ (300 mg)	Na_2SO_4 (300 mg)	CaH_2 (300 mg)	Silica Gel (1g)	Sephadex (1g)	Mol. sieve 3A (10 g)
				% Recovery			
α-Pinene	100	5	95	45	0	95	0
Myrcene	100	< 5	95	< 5	0	90	0
γ-Terpinene	95	0	100	< 5	0	90	0
p-Cymene	100	0	95	5	0	95	0
Caryphyllene	< 5	0	10	0	0	0	0
Linalool	0	0	10	0	0	0	0
β-Bisabolene	< 5	0	6	0	0	0	0
Carotol	0	0	10	0	0	0	0
				% Removal			
Water	100	100	80	100	--	--	--

Table 3. Percent recovery of carrot seed oil volatiles from and percent removal of water by potassium carbonate at various temperatures.

Compound	Pre-column Temperature			
	25°C	35°C	50°C	70°C
	% Recovery			
α-Pinene	100	100	100	100
Myrcene	100	95	100	92
γ-Terpinene	95	100	100	95
p-Cymene	100	100	100	100
Caryophyllene	< 5	15	30	62
Linalool	0	6	8	17
β-Bisabolene	< 5	10	18	50
Carotol	0	< 5	10	15
	% Removal			
Water	100	99.5	95	60

problem. Some typical results are illustrated in Table 4. Only potassium carbonate and calcium hydride proved satisfactory in these studies. Both of these desiccants permitted essentially 100% recovery of monoterpene hydrocarbons and a low recovery of higher boiling compounds while removing 99% or more of the water. There are no results reported for water removal by molecular sieve, sephadex or silica gel as these desiccants were not considered worthy of further investigation. Calcium carbide gave results similar to calcium hydride, however the hydride was given preference as the carbide contained impurities (background peaks) and apparently induced isomerization, rearrangements and/or formation of new compounds at higher temperatures (at temperatures in the order of 200°C, existing peaks increased and/or decreased in size, and new peaks appeared on the gas chromatograms). Table 4 illustrates a temperature of 200°C was necessary to recover the higher boilers in varying degrees from the calcium hydride. Although calcium hydride was the best drying agent, at any temperature (100% removal of water), at 200°C there appeared to be formation of p-cymene (150% recovery) and a few new peaks appeared mainly in the sesquiterpene region. Recoveries in excess of 100% of p-cymene were also noted when calcium sulfate and molecular sieves were heated. As Wrolstad and Jennings (1965) report p-cymene can be formed from monoterpene hydrocarbons by isomerization and oxidation, this type of synthesis may be occurring

Table 4. Percent recovery of carrot seed oil volatiles from and percent removal of water by various desiccants.

Compound	Drying Agent				
	K_2CO_3 (300 mg)	Mol Sieve 3A	$CaSO_4$ (300 mg)	CaH_2 (300 mg)	CaH_2 (300 mg)
	Pre-column Temperature				
	50°C	180°C	90°C	150°C	200°C
	% Recovery				
α-Pinene	100	15	20	100	100
Myrcene	95	15	50	80	80
γ-Terpinene	95	0	50	85	70
p-Cymene	100	20	140	100	150
Caryophyllene	30	0	15	12	60
Linalool	10	0	10	0	< 5
β-Bisabolene	25	0	10	10	25
Carotol	< 5	0	15	0	10
	% Removal				
Water	95		60	100	100

here. It is also foreseeable that conditions of heat and dehydration could convert alcohols to hydrocarbons. Dehydration of alcohols under these conditions may have contributed to the low recovery of alcohols.

Potassium carbonate when warmed to 35°C was concluded to be the best compromise, as 99.5% of the water was removed and the entire range of compounds were recovered to varying degrees; the monoterpene hydrocarbons being essentially 100% recovered and the higher boiling compounds being recovered in low percentages. The remaining 50 μg of water (99.5% of the water was removed) was eluted off the column as a fairly sharp peak. Potassium carbonate has the advantage of being a mild treatment (35°C), neutral, inexpensive and it can be regenerated as a drying agent by heating at 135°C. The potassium carbonate pre-column was especially useful in obtaining mass spectra of the monoterpene hydrocarbons. However, as there were minimal amounts of the highest boiling compounds, particularly polar compounds, recovered from the drying agent, the EC-1 inlet valve on the MS was utilized in obtaining spectra of these compounds. Volatiles were trapped by the headspace system in the absence of the pre-column and the EC-1 valve used to vent out excess water prior to the elution of the desired higher boiling compounds from the GLC column. The EC-1 valve was also used for examining the "lower boiling" compounds (with boiling points less than ethanol)

by GLC-MS, as on a TRIS column the water peak was eluted after ethanol.

Qualitative Analysis of Carrot Volatile Constituents

A qualitative study identifying the volatiles present in the raw carrot was necessary for examining the enzymatic formation of carrot aroma and for subsequent studies on the influence of variety, maturity and processing on carrot volatile constituents.

GLC-MS Analysis

GLC-MS was used to analyze the headspace volatiles present in aqueous extracts of raw carrots. GLC relative retention time data and comparison of mass spectra with tabulated standard spectra were used for identification of the compounds. The "lower boiling" compounds were separated on a TRIS column when chromatographed isothermally at 37°C. (This separation is illustrated in the chromatogram separating the "lower boiling" volatiles of canned carrots, Figure 11.) The retention data are recorded relative to the retention time of acetone which was assigned a value of 1.00. Acetone and methanol were not resolved on this column, however, they were resolved on Carbowax 20 M (Table 5). The component identifications are listed in Table 13. GLC confirmation (+) indicates that relative retention times for authentic compounds were within five percent of the

values for the compounds in the carrot extract. Aroma (+) indicates the aroma of the peak concerned is the same as the aroma of the authentic compound.

Figure 5 is a chromatogram of the "higher boiling" components when chromatographed on a Carbowax 20 M column. The column was held isothermally at 75°C for 17 min followed by a program of 4°/min up to a temperature of 195°C which was held.

Component identifications are listed in Table 5. As temperature programming over a wide range was employed the retention time data are recorded relative to two compounds, limonene and caryophyllene. Table 6 contains relative retention time data for the higher boiling components when chromatographed on an SF96-50 column which was programmed at 4°/min from 80°C to 200°C and held isothermally.

Tentative mass spectral identifications are given where spectra were either very weak or were of a mixture of more than one compound so that a positive match with reference spectra was not possible.

Significance of Qualitative Results

The only previous report on the characterization of volatile constituents in raw carrots (Buttery et al., 1968) failed to reveal the "lower boiling" compounds, diethyl ether, acetaldehyde, propanal, acetone, methanol and ethanol, reported in this study. These authors used conventional methods of steam distillation and extraction and the

Table 5. GLC-MS identification of compounds present in raw carrots, using a Carbowax 20 M column. Gas chromatogram is shown in Figure 5.

Peak no.	Compound	tr/tr Limonene	M.S. identification	M.S. reference	GLC confirmation	Aroma
1	Acetaldehyde	0.19	+	Cornu & Massot	+	+
2	Acetone	0.25	+	(1966)	+	
	Propanal		+	ibid	+	
3	Methanol	0.30	+	ibid	+	
4	Ethanol	0.35	+	ibid	+	
5	α-Pinene	0.46	+	Ryhage & von Sydow	+	
6	Camphene	0.57	+	(1963)	+	
7	β-Pinene	0.69	+	ibid	+	+
8	Sabinene	0.76	+	ibid	+	+
9A	Myrcene	0.81	+	ibid	+	+
9B	α-Phellandrene	0.88	+	ibid	+	
10	Limonene	1.00	+	ibid	+	+
11A	γ-Terpinene	1.21	+	ibid	+	+
11B	p-Cymene	1.38	+	ibid	+	+
12	Terpinolene	1.56	+	ASTM (1969)	+	+
13	Octanal	1.70	+	ibid	+	+

Continued

Table 5 Continued.

Peak no.	Compound	tr/tr Caryophyllene	M.S. identification	M.S. reference	GLC confirmation	Aroma
14	Unknown	0.70				
15	Unknown	0.75				
16	Unknown	0.77				
17	Unknown	0.83				
18	2-Decenal	0.85	Tentative	ASTM (1969)	+	
19	Unknown	0.88				
20	Unknown	0.93				
21	Bornyl acetate	0.96	+	ibid	+	+
22	Caryophyllene	1.00	+	ibid	+	+
23	Terpinene-4-ol	1.11	+	ibid	+	
	Sesquiterpene		+	ibid		
24	β-Bisabolene	1.14	+	Libbey (1969), Buttery et al. (1968)	+	+
25	γ-Bisabolene	1.17	+	ibid	+	+
26	Unknown	1.20				
27	Sesquiterpene	1.24	+	ASTM (1969)		
28	Sesquiterpene	1.27	+	ibid		
29	Aromatic M.W. 134	1.30	+	ibid		
30	Sesquiterpene	1.35	+	ibid		
31	Carotol	1.42	+	Libbey (1969), Buttery et al. (1968)	+	
32	Unknown					
33	Myristicin	1.74			+	+

Table 6. GLC-MS identification of compounds present in raw carrots, using an SF96-50 column.

Compound	tr/tr	M.S. identification	M.S. reference	GLC confirmation	Aroma
Acetaldehyde	0.14[a]	+	Cornu & Massot (1966)	+	+
Acetone	0.19[a]	+	ibid	+	
Ethanol	0.26[a]	+	ibid	+	
α-Pinene	0.7 [a]	+	Ryhage & von Sydow	+	+
Campene	0.77[a]	+	(1963)	+	
β-Pinene	0.84[a]	+	ibid	+	+
Sabinene	0.84[a]	+	ibid	+	+
Myrcene	0.89[a]	+	ibid	+	+
α-Phellandrene	0.93[a]	+	ibid	+	
Limonene	1.00	+	ibid	+	+
γ-Terpinene	1.10[a]	+	ibid	+	+
p-Cymene	1.18[a]	+	ibid	+	+
Octanal	1.17[a]	Tentative	ibid	+	+
Terpinolene	1.20[a]	+	ASTM (1969)	+	+
Terpinene-4-ol	0.82[b]	+	ibid	+	+
Caryophyllene	1.00	+	ibid	+	+
β-Bisabolene	1.14[b]	+	Libbey (1969), Buttery et al. (1968)	+	+
γ-Bisabolene	1.18[b]	+	ibid	+	+

[a] tr/tr relative to limonene

[b] tr/tr relative to caryophyllene

"lower boiling" compounds could have been lost due to elevated temperatures over long periods or during the necessary evaporation and concentration of solvent steps.

With the exception of the presence of α-phellandrene the monoterpenes and sesquiterpenes identified confirm Buttery et al.'s (1968) findings using a different method of sample preparation and analysis. In contrast to Buttery et al.'s (1968) finding of several long chain aldehydes (octanal, nonanal, 2-nonenal, 2-decenal, 2,4-decadienal and dodecanal) only octanal and 2-decenal were found in this study and in very small amounts. The milder, inert gas entrainment procedure may have minimized formation of these aldehydes which can be formed from C_{18} unsaturated fatty acids by autoxidation (Schultz, Day and Sinnhuber, 1962), and by heating (Buttery et al., 1968). Carrots have a high essential fatty acid content (Dalgarno and Birt, 1963). The headspace system also has the advantage of being convenient and rapid, however, with the classes of compounds involved in this study it does have the disadvantage of incomplete separation of some oxygenated compounds and sesquiterpenes in the "higher boiling" region. For instance, terpinene-4-ol and an unidentified sesquiterpene have the same retention time under these conditions of analysis. The on-column entrainment system did not permit the separation of sesquiterpene hydrocarbons and oxygenated components by column chromatography as used by Buttery et al. (1968). It is

possible trace amounts of longer chain aldehydes could have been present and remained undetected.

The characteristic raw carrot aroma is difficult to define but from the observations of the author and of others it contains, to varying degrees, "soft", "sweet", "pumpkin-like" notes, "perfumy" notes and predominantly a stronger (green, earthy) "carrot tops" note. From sensory evaluation of column effluents (using effluent splitters) there would appear to be several chromatographic regions (compounds) contributing to the raw carrot aroma. These include acetaldehyde which contributes a soft, "sweet" note and sabinene and myrcene; both of these, the myrcene in particular, contributing "green", "earthy", "carrot-top" notes. Terpinolene ("perfumy") and to a lesser extent caryophyllene and carotol ("soft" notes) are also believed to contribute character-impact notes. Acetaldehyde has been shown to be important in several foods (Amerine, 1964). Acetaldehyde can be detected in water at less than 1 ppm (Sheldon, 1968) and all of these terpenes are present in concentrations far in excess of their reported thresholds in aqueous solution (Buttery et al., 1968). A soft squash-like (cucumber) aroma was detected in the vicinity of caryophyllene on Carbowax 20 M. The retention time on Carbowax 20 M corresponded to that of the potent odorant 2, 6-nonadienal (odor of cucumbers, Forss et al., 1962). The retention time of the aroma and of 2, 6-nonadienal also coincided on SF96-50 where the retention times of

2, 6-nonadienal and caryophyllene were not coincident. However, there was an insufficient quantity of the compound present to permit further characterization of the compound. Buttery et al. (1968) also found tentative evidence for the presence of this compound.

Obviously carrot has a complex flavor profile. No single compound was found that could be claimed solely responsible for the characteristic raw carrot flavor (aroma), therefore it is likely carrot flavor is an expression of a combination of compounds. These studies indicate character-impact compounds would include acetaldehyde, sabinene, myrcene, terpinolene, caryophyllene and carotol.

Enzymatic Regeneration of Carrot Aroma

Owing to the elusive nature of the compound(s) responsible for raw carrot aroma an investigation using the "flavorese" concept appeared an alternative approach for elucidating what compound(s) were responsible for carrot flavor. Preliminary experiments using acetone powders (A) and substrates ("precursor" material) prepared from carrots confirmed this concept and further investigation of what volatiles were being released enzymatically offered an attractive system for studying the biosynthesis of a flavor and determining what compound(s) were responsible for carrot aroma.

Early experiments involved the use of 5 g of reconstituted freeze dried precursor incubated for 90 min at 37°C with 50 mg of acetone

powder A, followed by organoleptic evaluation. As described in the experimental section, four samples were prepared: the above enzyme-precursor reaction sample and three controls, enzyme only, precursor only and heat inactivated enzyme and precursor. At the levels of enzyme used the enzyme only controls were odorless and did not contribute a background aroma. The precursor only control was also essentially odorless but had a bland, "hay-like" aroma. Characteristic raw carrot aromas were only developed in the enzyme-precursor samples. Panel member descriptions included "carroty", "fresher", and "raw carrot". These studies indicated it was only necessary to prepare the enzyme-precursor and precursor only samples. When judges were presented with three coded samples in triangular tests, one of enzyme-precursor and two of precursor only, in 90% or greater of the cases the enzyme treated sample was detected as different and described as carrot. The fact that precursor treated with heat inactivated enzyme did not develop a raw carrot aroma in any instance is regarded as good evidence that the process is enzymatic in nature. However, these results were not always reproducible. Acetone powders prepared by the same method as described for Powder A were 'active' sometimes and only weakly active or inactive in other instances. In an attempt to obtain enzyme extracts that were reproducibly active several methods of enzyme preparation were investigated. As enzyme preparations that were

essentially odorless (to minimize background interference) and in a form convenient for storage were desirable, the preparation of acetone powders was preferred. Cell free extracts investigated had background carrot aromas and were not suitable. Aqueous carrot extracts were observed to undergo "browning" indicating oxidation of phenolic compounds. A study on the oxidative browning of carrots was reported by Chubey (1966). As phenolics are reported to interfere with the isolation of and inhibit the activity of plant enzymes (Loomis, 1968), enzyme preparation methods were designed to minimize or prevent this. These included the use of PVP (Loomis and Battaile, 1966) PEG and direct acetone extraction (Loomis, 1968). However, these acetone powders when reacted with precursor gave similar results to those obtained by using the regular acetone powder (A). Reactions were carried out at pHs ranging from 5-8, and in the presence of 0.01 M EDTA (the possibility of metal ion inhibition existed) with no noticeable improvement in the results. An enzyme preparation prepared by ammonium sulfate precipitation using the method described by Manners and Rowe (1968) was inactive. Three different substrate sources were examined, freeze dried precursor, nitrogen purged precursor and a cooked carrot precursor preparation. Based on sensory evaluation, freeze dried precursor prepared with a three hour distillation under reduced pressure was the most active, followed by the nitrogen purged precursor and freeze dried precursor

prepared by an exhaustive distillation for eight hours. The cooked precursor was inactive. With the exception of the ammonium sulfate preparation, all the enzyme preparations were capable of erratic "flavorese" activity but no preparation was found to be consistently active. Based on sensory analysis acetone powders (D) produced the best activity, followed by powders (A), (C), and (B) in descending order of activity. The lack of uniformity in the results is disappointing but perhaps not as unreasonable as they first appear when one considers how complex and broad the system under investigation is (Reed, 1966; Hewitt, 1963), and the fact that previous workers in the field have also reported similar problems (Miller, 1958).

Enzyme activity evaluated solely on the basis of sensory analysis is somewhat subjective. The volatiles released in enzyme reaction mixtures and controls were also evaluated objectively using the headspace analysis system and conditions previously described. Enzyme reaction samples possessing regenerated carrot aroma and control samples were examined. Figure 6 is typical of the results obtained using freeze dried "precursor" (prepared with eight hours distillation) and acetone powder (D). A range of X1 on the gas chromatograph was used and a 3:1 effluent splitter to permit simultaneous odor evaluation of the peaks recorded. The enzyme only control is not included as apart from the presence of an acetone peak, it had no detectable background. The substrate only chromatogram

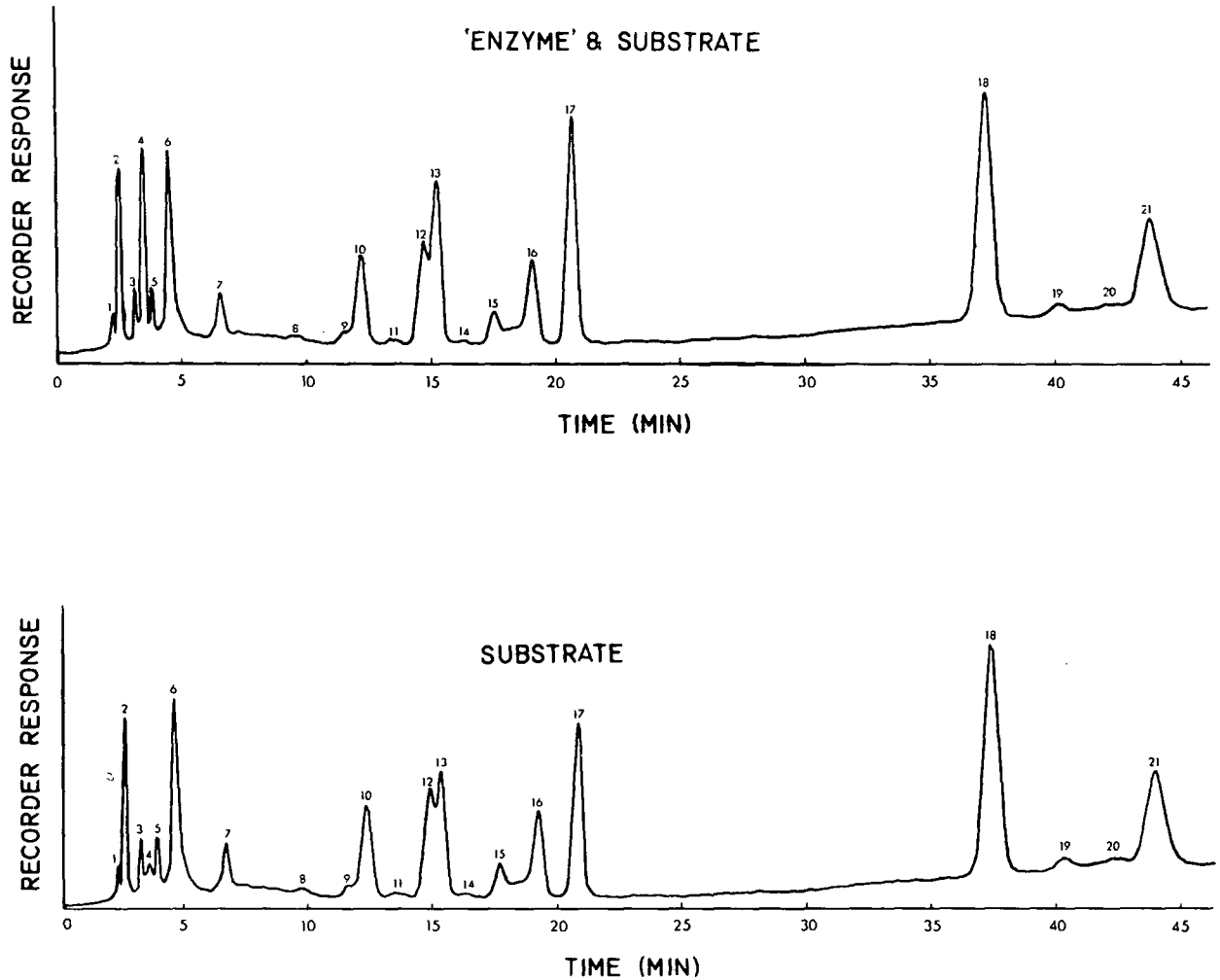

Figure 6. Analysis of the headspace volatiles present in substrate (vacuum distilled for 8 hours, freeze dried) and enzyme treated substrate samples, using an SF96-50 column. (Peak no. 2, acetaldehyde; 4, acetone; 6, ethanol; 10, α-pinene; 12, β-pinene and sabinene, 13, myrcene; 15, limonene; 16, γ-terpinene; 17, terpinolene; 18, caryophyllene; 21, γ-bisabolene.)

shows the extent to which volatiles are removed with reduced pressure steam distillation (for eight hours) followed by freeze drying. The only major difference between the two chromatograms is the presence of a background acetone peak (peak no. 4) contributed by the acetone powder, appearing in the enzyme treated substrate chromatogram. In an attempt to exclude the possibility of enzyme induced peak(s) being present but masked by one of the background peaks, the separation of compounds was examined on three different columns, Carbowax 20 M, SF96-50 and TRIS. In each instance enzyme reaction samples and duplicate controls were examined. The appearance of new enzyme induced compound(s) and/or significant increases in existing compounds, coinciding with the regeneration of raw carrot aroma, was not detected. As the possibility of a masked peak still existed, attempts at preparing an active substrate with essentially no detectable background were investigated. A nitrogen purged precursor material, prepared as described in the experimental section, was the closest to fulfilling these needs. Figure 7 illustrates the extent to which the background was reduced when examined at range X1 with a 3:1 splitter on a Carbowax column. Essentially only the higher boiling compounds that were originally present in large amounts (e.g. peak no. 5, caryophyllene and peak no. 8, γ-bisabolene), remain. However, the enzyme treated substrate was devoid of any new peaks or increases in existing peaks. These

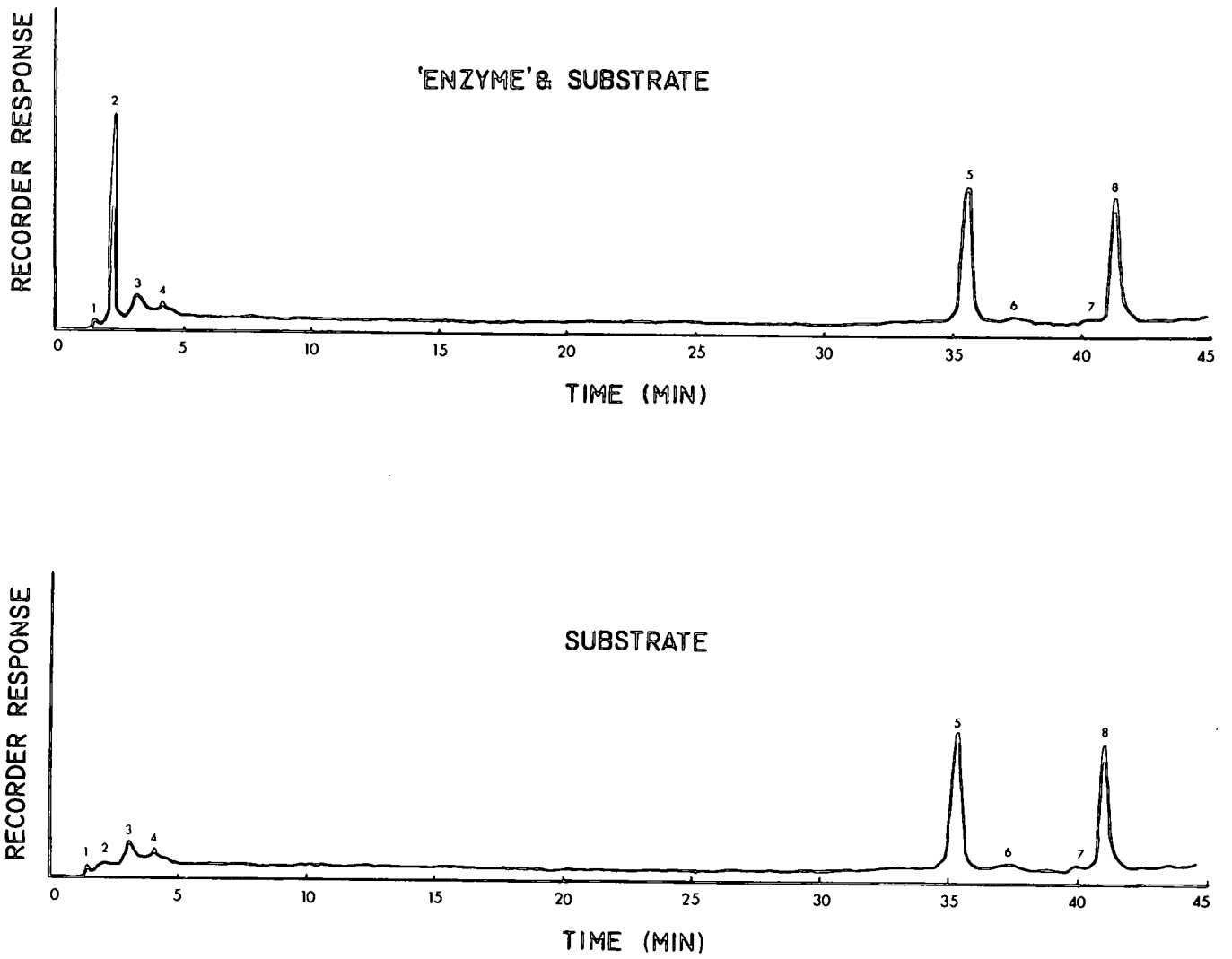

Figure 7. Analysis of the headspace volatiles present in substrate (nitrogen purged) and enzyme treated substrate samples, using a Carbowax 20 M column. (Peak no. 1, acetaldehyde; 2, acetone; 3, ethanol; 4, α-pinene; 5, caryophyllene; 6, γ-bisabolene.)

results would indicate that the compound or compounds responsible for carrot aroma are beyond the limits of detection of the method. This would not be unreasonable as several compounds important in flavor have been reported with aroma thresholds well beyond the limits of instrumental detection. For instance the recent report by Buttery et al. (1969) of 2-methoxy-3-isobutyl-pyrazine which has a bell pepper aroma at concentrations of a few parts per trillion. Also, although the headspace analysis system used did provide a good recovery of a wide range of compounds, if the compound responsible for carrot aroma was a very high boiling compound, particularly a polar compound, it may not have been recovered from solution by this method.

A freeze dried precursor sample was also prepared using the less exhaustive reduced pressure steam distillation conditions of three hours at approximately 1 mm pressure. This substrate, upon reconstitution, did possess a weak carrot-like aroma and was therefore not as good a control. The background (Figure 8) is several fold that obtained by distilling for eight hours. However, when treated with suitable enzyme preparations a stronger characteristic raw carrot aroma than achieved with the other freeze dried substate was induced. When the headspace volatiles were examined there were no new compound(s) formed and no significant changes in concentration of existing compounds. In these experiments as in the earlier

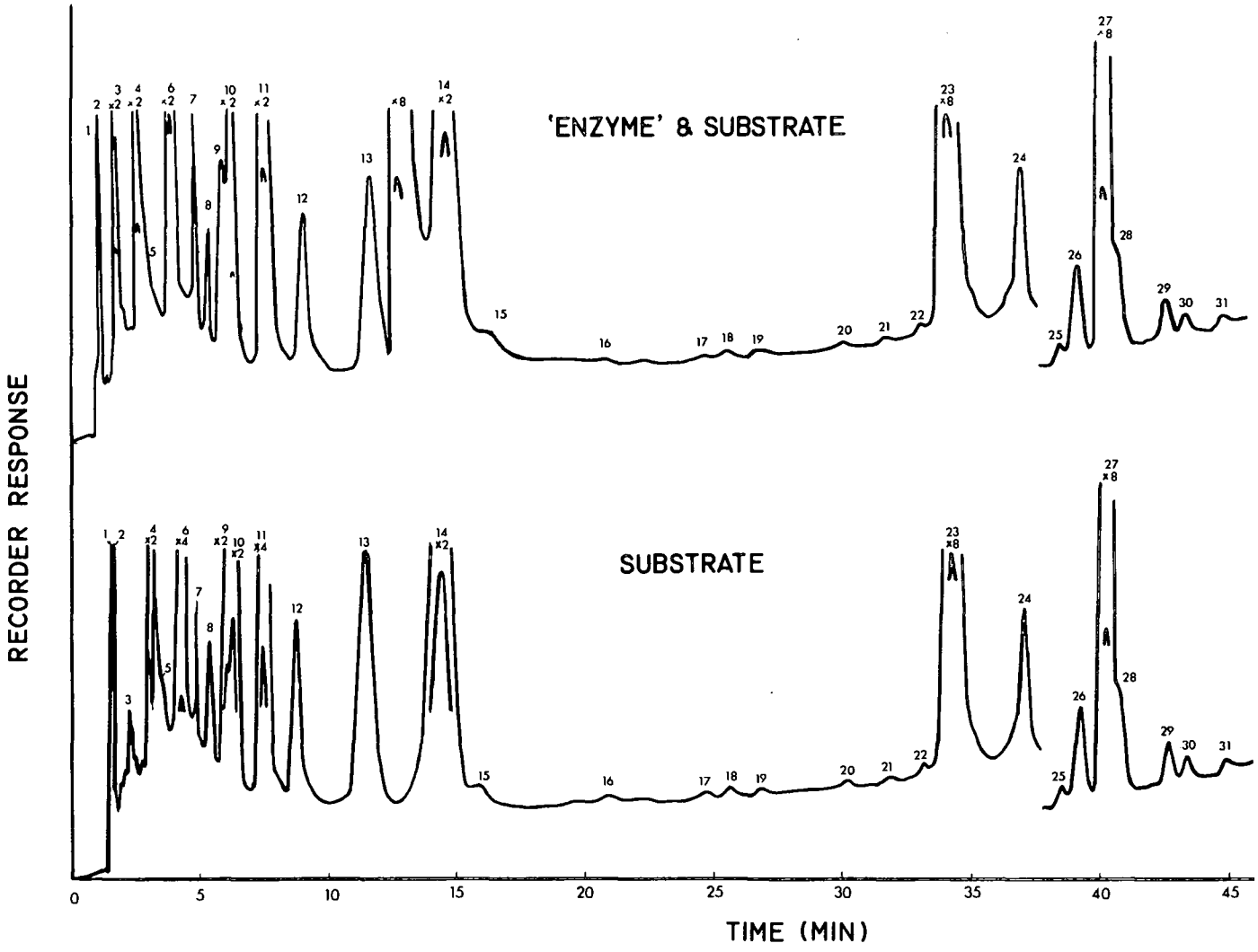

Figure 8. Analysis of the headspace volatiles present in substrate (vacuum distilled for 3 hours, freeze dried) and enzyme treated substrate samples, using a Carbowax 20 M column.

experiments using freeze dried precursor material the duplicate controls were generally reproducible to ± 10%, however, periodically the concentration of an individual compound could vary by as much as ± 25%.[5] Because of this the limits of reproducibility are set at ± 25%, however it is possible enzymatically induced increases in the order of 50% may have occurred for a given compound. Increases of this magnitude for α-pinene, sabinene, myrcene and terpinolene were frequently observed and may have been real enzyme increases but owing to the inherent variation in volatile content of the controls the results must be interpreted with extreme care. Even if these increases represented enzyme activity they are not believed to be responsible for the induced carrot aroma, as in some instances the concentration of induced compound was below the reported threshold for detection[6] and on several occasions there were no detectable increases of these compounds coinciding with the induced characteristic carrot aroma. The possibility exists that rather than synthesis occurring an enzymatic or chemical hydrolysis may result in an irregular release of volatiles, for instance by glycosidase action or from polysaccharide encapsulation.

Figure 8 illustrates the background present with freeze dried

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[5] Refer to quantitative analysis of carrot volatile constituents.

[6] Refer to quantitative analysis of carrot volatiles.

precursor prepared by steam distilling for three hours. It also demonstrates the appearance of a large peak (between γ -terpinene, peak no. 13 and terpinolene, peak no. 14) in the enzyme treated substrate which had a retention time and odor corresponding with p-cymene. The enzyme preparation used in this instance was an acetone powder (A) using Autumn King carrots, and induced a very strong raw carrot aroma. Apart from the appearance of the new peak the substrate and enzyme treated substrate chromatograms are essentially identical. Although this result was reproduced in three consecutive experiments using the same enzyme and precursor materials, it was not reproduced at any other time during this study, using the same or different enzyme and precursor preparations. A compound such as p-cymene is readily formed by isomerization of other monoterpene hydrocarbons (Wrolstad and Jennings, 1965). However, a corresponding decrease in other monoterpenes was not apparent. The possibility also exists that the compound p-cymene was being formed from was so unstable and transient under conditions of analysis that it was not detected. It is quite possible a compound that is unstable under the conditions of analysis is responsible for fresh carrot aroma. Recently Varo and Heinz (1969) reported a terpene aldehyde, 1, 4-p-methadiene-7 al, believed important in cumin odor, that was highly reactive and unstable during analysis conditions, undergoing rearrangement and disproportionation

reactions to produce the aromatic terpene aldehyde, cumin aldehyde (as well as other related compounds).

Owing to the inconclusive and irreproducible nature of the results with the enzyme studies this aspect of the investigation into characterizing carrot flavor was terminated and the methods developed applied to investigating other problems concerning carrot volatiles, namely the influence of variety, maturity and processing on the carrot volatile constituents. However, some useful conclusions can be drawn from these studies. The compound(s) responsible for carrot aroma remains elusive and may be a potent compound, present in very low concentrations beyond the limits of detection of the method used, or very labile, and unstable under the analysis conditions. Of course, the possibility also exists that carrot aroma results from a complex interaction of several compounds.

Quantitative Analysis of Carrot Volatile Constituents

To gain an understanding of an aroma it is necessary to know not only which compounds are present but also the quantity of individual volatiles present. The concentration of compounds present in aqueous carrot extracts were estimated from standard curves of peak area versus concentration and are reported in parts per million (ppm) and expressed on a fresh weight basis. (A dilution factor of 1:1 occurring during the aqueous extraction is corrected for in the

standard curves.) Methanol and acetone were not resolved by GLC on the TRIS column but were resolved by the Carbowax 20 M column. In contrast, acetone and propanal were not resolved on Carbowax 20 M but were separated by the TRIS. By using both columns the concentration of each of these compounds could be determined. Typical standard curves are shown in Figures 9 and 10.

Quantitative Analysis of Raw Carrots

The results in Tables 7 and 8 are typical for those obtained for three consecutive analysis of three different batches of equal size carrots of the same variety (Autumn King) harvested at the same time (20 weeks from planting date). Whereas the concentration of lower boiling compounds, apart from ethanol, are fairly reproducible, there is considerable variation in concentration of individual compounds in the "higher boiling" fraction. The total essential oil concentration only varied from 24-28 ppm, however, the concentration of individual compounds could vary by as much as a factor of two or three in some instances (for example α-pinene, sabinene, limonene). As the analytical method had proven very reproducible in model system studies using carrot seed oil and as the material being analyzed was subjected to identical conditions this variation is interpreted as representing inherent variation of the volatiles within the source material. Undoubtedly enzymatic activity is occurring during the aqueous

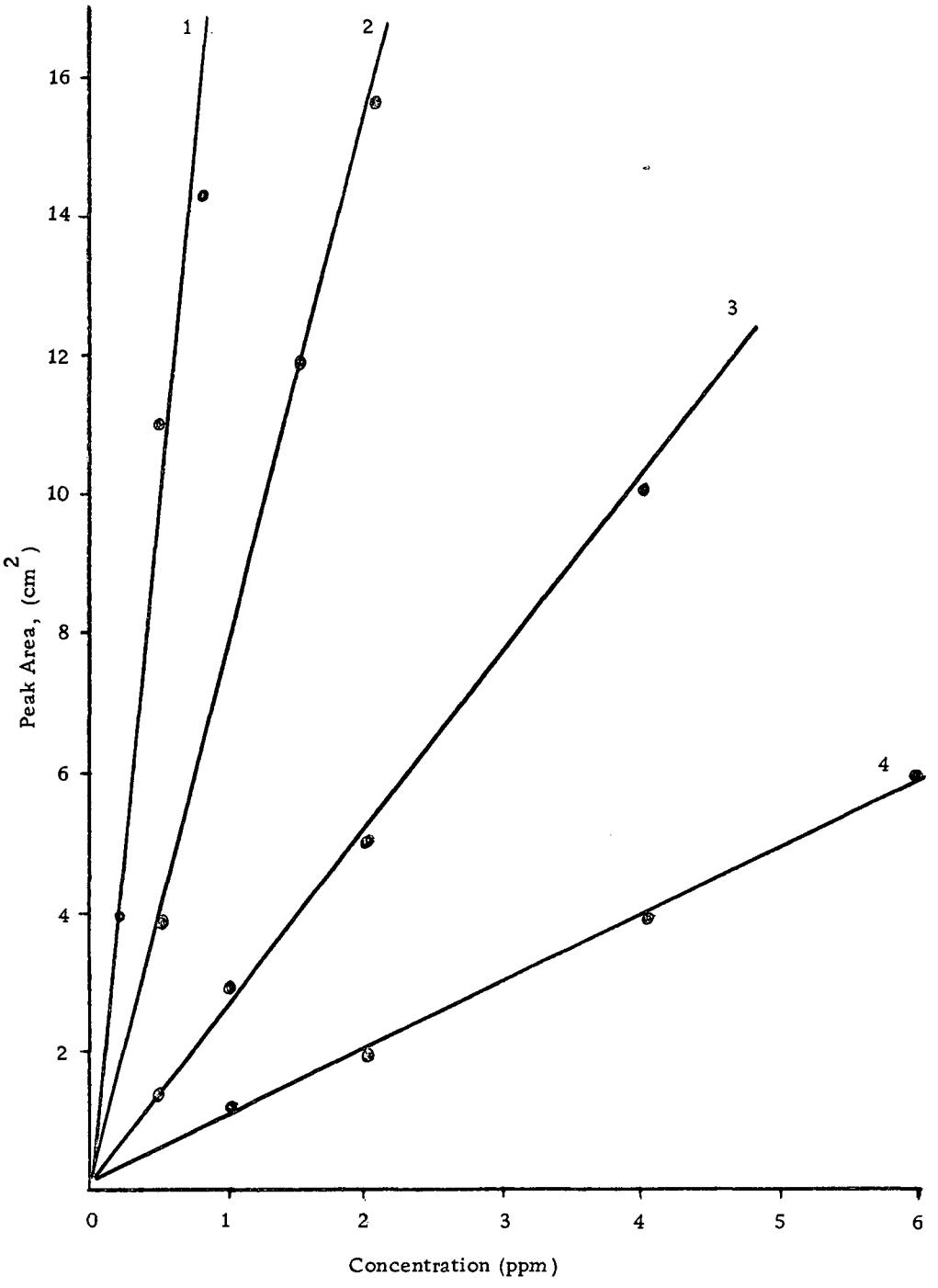

Figure 9. Peak areas obtained from various concentrations of "lower boiling" compounds. (1) Dimethyl sulfide, (2) Propanal, (3) Acetone, (4) Ethanol

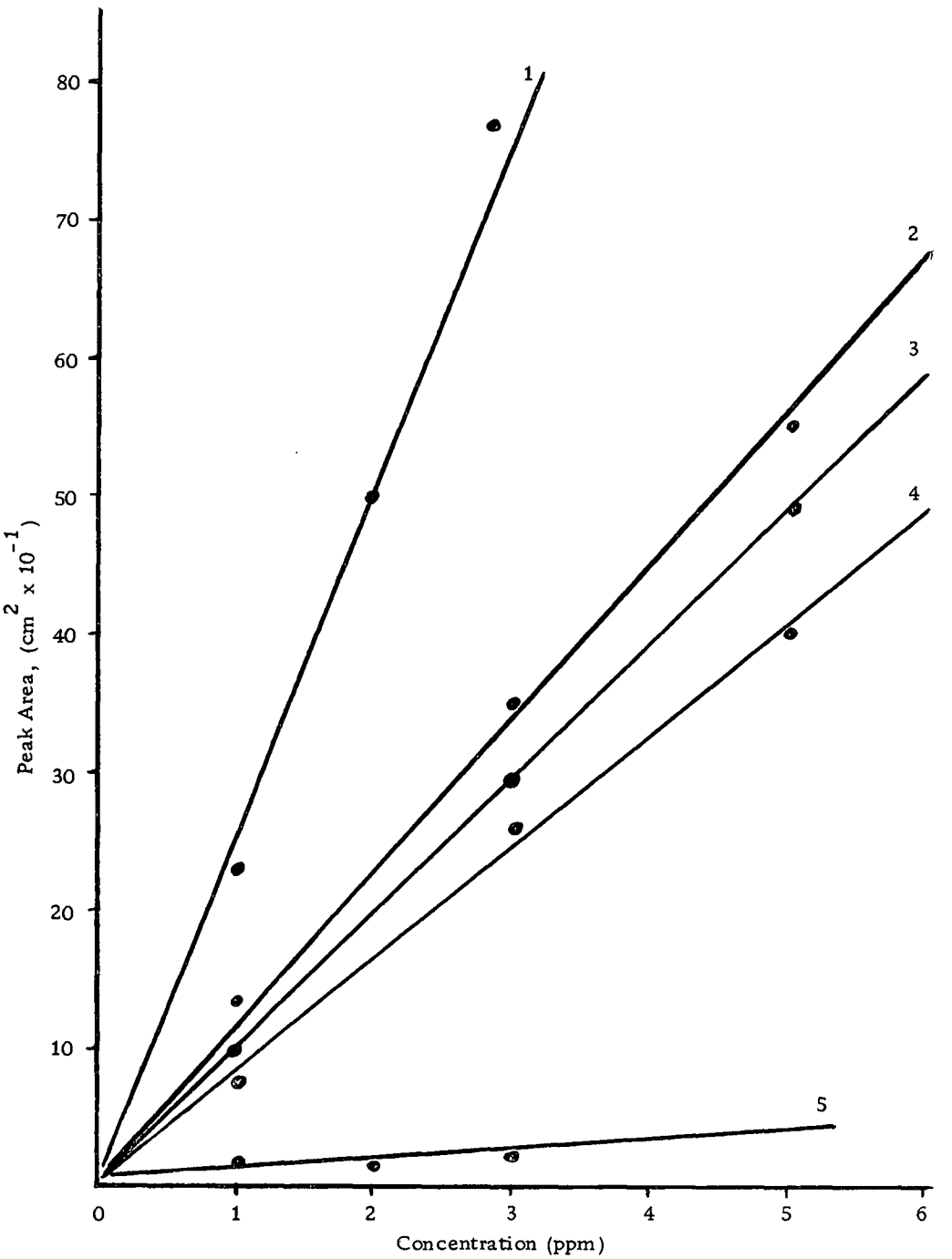

Figure 10. Peak areas obtained from various concentrations of "higher boiling" compounds. (1) α-pinene, (2) Terpinolene, (3) Octanal, (4) Caryophyllene, (5) Carotol.

Table 7. Intravarietal variation of the "lower boiling" carrot volatile constituents.

Compound	Analysis I	Analysis II	Analysis III
Ether[a]	Tr.[b]	Tr.	Tr.
Acetaldehyde[a]	0.75	0.60	0.45
Acetone[a]	0.20	0.20	0.31
Propanal[a]	Tr.	Tr.	Tr.
Methanol[a]	0.04	0.03	Tr.
Ethanol[a]	80.00	65.00	5.00

[a]Concentration in ppm

[b]Trace

Table 8. Intravarietal variation of the "higher boiling" carrot volatile constituents.

Compound[a]	Analysis I	Analysis II	Analysis III
α-Pinene	0.79	1.40	1.35
Camphene	0.14	0.12	0.12
β-Pinene	0.36	0.14	0.21
Sabinene	5.24	2.60	2.65
Myrcene	4.65	4.45	4.40
α-Phellandrene	Tr.[b]	Tr.	Tr.
Limonene	0.66	0.72	1.62
γ-Terpinene	0.23	0.22	0.42
p-Cymene	0.37	0.25	0.50
Terpinolene	5.52	3.90	4.15
Octanal	Tr.	0.02	0.02
2-Decenal	0.02	0.01	Tr.
Bornylacetate	0.11	0.08	0.20
Caryophyllene	5.00	4.30	6.80
Terpinene-4-ol	--	--	--
β-Bisabolene	0.34	0.44	0.25
γ-Bisabolene	4.85	4.45	2.40
Carotol	0.20	0.16	0.20
Total	28.00	24.00	25.00

[a]Concentration in ppm

[b]Trace

extraction. Consecutive analysis of identical aliquots from the same aqueous extract, one sample analyzed immediately after extraction and the other after standing two hours at room temperature produced reproducible results (within 10% variation of the mean). This would indicate that any enzyme action that may be occurring is at least constant and apparently to completion. However, as a similar intravarietal variation also occurred in aqueous samples prepared from canned carrots where the enzymes have been inactivated, the variation was regarded as representing different levels of metabolism within the carrot source material. This is not unreasonable in the light of recent evidence suggesting monoterpenes are subject to rapid metabolic turnover in plant tissue (Burbott and Loomis, 1968). These authors also reported variations as great as two fold in the monoterpene essential oil content of simultaneously harvested samples of peppermint leaves of the same size. Other chemical constituents in carrots have also been found subject to intravarietal variation (Carlton and Peterson, 1963). Sistrunk, Bradley and Smith (1967) and Bradley et al. (1967) reported several pre-harvest factors such as sunlight, soil, temperature and moisture greatly influenced sugar, pectin and carotenoid concentrations in the carrot. Undoubtedly these factors are also influencing the concentration of volatiles in carrots. In all subsequent quantitative studies the concentrations of individual compounds are reported as one figure

representing an average of not less than two independent determinations.

The Influence of Maturity, Variety, and Storage on Carrot Volatile Constituents

To examine the influence of maturity, Wisconsin 5 and Autumn King varieties were harvested and analyzed at approximately monthly intervals from the date of planting (June 12, 1969). It is difficult to determine an index of carrot maturity as the tissue has continuous cell division and growth. In this study, age, size and weight are the parameters used as a guide to maturity. Great care must be taken in interpreting these results owing to the inherent variation in volatile contents within a given sample of carrots. Only broad trends are considered meaningful.

The results in Table 9 indicate that whereas the concentration of acetone, propanal and methanol remain relatively constant throughout the study the acetaldehyde and particularly the ethanol concentration increases dramatically in the late season (fall, winter) carrots. The ethanol increases from a few ppm to over 1000 ppm. Accumulation of acetaldehyde and ethanol indicates anaerobic respiration. Accumulation of these compounds by plant tissues subjected to stresses such as low oxygen supply is well documented (Cossins and Beevers, 1963; Amerine, 1964). Fall and winter in Oregon are

Table 9. Influence of maturity on the "lower boiling" volatiles in carrots.

Compound[c]	Age[a]				
	6	16	20	24	28
	Average weight, g				
	20	120	150	150	150
	Average diameter, inches				
	0.4	1.0	1.5	1.5	1.5
Ethanethiol	0.00	0.00	0.00	0.00	0.00
Diethyl ether	Tr.[b]	Tr.	Tr.	Tr.	Tr.
Dimethyl sulfide	0.00	0.00	0.00	0.00	0.00
Acetaldehyde	0.15	0.20	0.75	1.40	1.82
Acetone	0.10	0.30	0.25	0.15	0.40
Propanal	Tr.	Tr.	Tr.	Tr.	Tr.
Methanol	0.05	0.06	0.04	0.10	0.05
Ethanol	1.50	35.00	42.00	140.00	1200.00

[a]Weeks from date of planting

[b]Trace

[c]Concentration in ppm

very wet seasons producing long periods of essentially "water-logged" soil conditions which could induce anaerobic metabolism in a storage root such as the carrot. Lemon (1962) and Lemon and Wiegand (1962) indicate root respiration can be limited by the diffusion of oxygen to the root surface. Poorly drained soils would greatly limit the diffusion of oxygen and the aeration conditions in the soil in general (Klock and Brooks, 1968).

The determination of ethanol concentration particularly in the early season carrots produced the most variable results of all the volatiles analyzed. Concentrations ranging from a few ppm to 40 ppm were detected in the early season carrots. Cossins and Beevers (1963) reported ethanol accumulated in periods of anaerobiosis can be metabolized when the tissue gains better access to oxygen. These authors found plant tissues, including carrot dics, rapidly metabolized labelled ethanol to carbon dioxide, organic acids, amino acids and other products, in periods of four hours or less. This evidence for rapid metabolic turnover of ethanol dependent on external stresses may account for the variation in ethanol content found in carrots as repeated analysis of the same carrot harvest could continue over a period of one day.

Examination of the higher boiling compounds in Table 10 indicates that although the total essential oil content remains relatively constant between 21 to 28 ppm for the first 20 weeks of growing

Table 10. Influence of maturity on the "higher boiling" volatiles in carrots.

Compound[c]	Age[a]				
	6	16	20	24	28
	Average weight, g				
	20	120	150	150	150
	Average diameter, inches				
	0.4	1.0	1.5	1.5	1.5
α-Pinene	0.65	1.00	0.25	0.65	1.18
Camphene	0.14	0.10	0.01	0.03	0.02
β-Pinene	0.46	0.07	0.65	0.61	0.55
Sabinene	0.45	0.08	2.31	0.75	1.00
Myrcene	1.30	2.90	1.28	2.30	0.61
α-Phellandrene	Tr.[b]	0.65	0.25	0.06	0.06
Limonene	0.52	0.10	0.29	0.55	0.60
γ-Terpinene	2.15	1.18	0.65	4.05	7.75
p-Cymene	0.00	0.00	0.00	0.00	Tr.
Terpinolene	5.45	3.68	3.10	13.00	7.75
Octanal	0.02	0.01	0.02	0.02	0.02
2-Decenal	0.02	Tr.	Tr.	0.00	0.00
Bornyl acetate	0.05	0.08	0.04	0.03	0.02
Caryophyllene	6.65	11.90	17.55	18.52	15.55
Terpinene-4-ol	--	--	--	--	--
β-Bisabolene	0.34	0.61	0.22	0.42	5.00
γ-Bisabolene	2.25	5.00	1.00	7.45	11.65
Carotol	0.15	Tr.	0.28	0.31	0.30
Total	21.00	27.00	28.00	49.00	50.00

[a]Weeks from date of planting [b]Trace [c]Concentration in ppm

season, the concentration of individual terpenes is constantly changing. This further confirms the belief that the terpenes are undergoing constant metabolic changes and are not merely being accumulated as an end-product. The total essential oil content increased to approximately 50 ppm at the end of the season (24-28 weeks) largely due to an apparent increase in the synthesis of the sesquiterpenes caryophyllene and bisabolene and to a lesser extent the monoterpenes γ-terpinene and terpinolene. The Autumn King variety gave similar trends with a lesser increase to approximately 35 ppm at the end of the season. As these results are expressed on a fresh weight basis the increase could in part reflect an anticipated decrease in moisture content with age. Similar variations in terpenes and an increase in monoterpene hydrocarbons with increasing maturity in citrus peel and leaf oils has been reported by Attaway, Pieringer and Barabas (1966).

Examination of Tables 11 and 12 illustrate the results obtained by comparing the volatile constituents present in six different carrot varieties, all harvested and analyzed within a three day period in November. The varieties were selected to represent a range of flavor (aroma) characteristics that had been observed in both the raw and canned carrots. The selections were based on preliminary studies on canned carrots involving preference panel subjective evaluations (Varseveld, 1969). It was observed that in some

Table 11. Influence of variety on the "lower boiling" carrot volatile constituents.

Compound[a]	Wisconsin 5	Imperator	Nantes	Chantenay	Oregon 4362	Autumn King
Ethane thiol	0.00	0.00	0.00	0.00	0.00	0.00
Diethyl ether	Tr.[b]	Tr.	Tr.	Tr.	Tr.	Tr.
Dimethyl sulfide	0.00	0.00	0.00	0.00	0.00	0.00
Acetaldehyde	0.75	0.60	0.85	0.66	0.80	0.40
Acetone	0.30	0.08	0.04	0.03	0.03	0.31
Propanal	Tr.	Tr.	0.02	Tr.	Tr.	Tr.
Methanol	0.06	Tr.	0.06	0.05	0.05	0.04
Ethanol	35.00	50.00	51.00	30.00	31.00	80.00

[a]Concentration in ppm

[b]Trace

Table 12. Influence of variety on the "higher boiling" carrot volatile constituents.

Compound[a]	Variety					
	Wisconsin 5	Imperator	Nantes	Chantenay	Oregon 4362	Autumn King
α-Pinene	1.00	0.09	1.10	0.65	0.05	0.79
Camphene	0.10	0.04	0.06	0.10	0.03	0.14
β-Pinene	0.07	0.05	0.22	0.33	0.09	0.36
Sabinene	0.08	0.10	0.68	0.68	0.13	3.24
Myrcene	2.90	0.35	0.32	1.85	0.06	4.65
α-Phellandrene	0.65	Tr.[b]	Tr.	0.06	0.03	Tr.
Limonene	0.10	0.46	0.19	0.70	0.12	0.06
γ-Terpinene	1.18	Tr.	0.75	3.51	0.22	0.03
p-Cymene	0.00	0.13	0.00	0.00	0.00	0.78
Terpinolene	5.45	6.05	0.81	5.45	2.45	4.52
Octanal	0.02	0.01	0.03	0.02	Tr.	Tr.
2-Decenal	Tr.	0.00	0.00	Tr.	0.00	Tr.
Bornyl acetate	0.05	0.22	0.02	0.12	Tr.	0.11
Caryophyllene	6.65	10.00	3.30	5.05	1.21	4.00
Terpinene-4-ol	--	--	--	--	--	--
β-Bisabolene	0.34	1.18	0.28	0.46	0.07	0.34
γ-Bisabolene	2.25	5.55	2.95	4.05	0.22	4.75
Carotol	0.15	0.22	0.38	Tr.	Tr.	0.10
Total	27.00	26.00	11.00	23.00	5.00	24.00

[a] Concentration in ppm

[b] Trace

instances the raw carrot aroma characteristics appeared to carry through to the canned product. For instance Autumn King was frequently described as having a strong, "green", "toppy" aroma, whereas Oregon 4362 was described as being mild and "perfumy" in aroma. Table 12 indicates the Autumn King variety has a relatively high concentration of the "green", "toppy" sabinene/myrcene compounds (approximately 30% of the essential oil throughout the growing season) which could account for the stronger nature of this carrots' aroma (flavor). In contrast, the mild, bland, "perfumy" Oregon 4362 not only has a relatively low concentration of these compounds (approximately 4% of the essential oil) but also has a relatively high concentration of the "perfumy" terpinolene (approximately 50% of the essential oil). Apart from the Oregon 4362 and the Nantes the total essential oil concentration of all varieties was reasonably constant between 24-27 ppm. The value of 5 ppm for the Oregon 4362 was dramatically lower than the other varieties and may be responsible for the much milder, blander nature of the carrot. Nantes with an essential oil concentration of 11 ppm was also noted as being a milder flavored carrot.

The other varieties displayed no markedly differing flavor characteristics. A somewhat similar variation with variety and harvest in the concentration of iso-amyl alcohol, pentanol and 3-hexenol-1 in tomatoes has been reported by Johnson et al. (1968).

These authors utilized the entirely different method of distillation and solvent extraction for preparing their flavor extracts.

The only qualitative difference found within the six varieties was the consistent appearance of up to 2 ppm of p-cymene in the Autumn King variety. Only on very rare occasions was its presence noted in other varieties and then only in trace amounts. This strong aromatic compound may also contribute to the stronger flavored Autumn King variety. However, the appearance of this compound may well be an artifact of analysis as Wrolstad and Jennings (1965) reported p-cymene could be readily formed from sabinene, α and γ-terpinene and α-phellandrene by isomerization and oxidation.

Varietal differences such as those cited may be of value to the plant geneticist who, by systematic breeding, could develop carrot varieties with more desirable flavors for both the fresh market and for processing. Stevens (1967, 1970) in studies involving oct-1-en-3-ol and linalool in the snap bean, and 2-isobutyl thiazole, methyl salicylate and eugenol in tomatoes, has demonstrated this type of study is possible. Quantitative differences in the concentration of these compounds were shown to be inheritable.

In Oregon most of the harvesting of carrots for processing occurs in November (corresponding to 20 weeks from planting in this study), however, harvesting may continue through December. Other quality factors aside, there would appear to be no disadvantage in

harvesting late season carrots for processing as far as volatile content is concerned.

During the course of this study carrot samples which had been stored in polyethylene bags were observed to undergo some interesting changes on storage. Five pound lots of carrots (Wisconsin 5, Autumn King) were washed, topped and sealed under a high relative humidity in polyethylene bags which were stored in a ventilated room, in the dark, at 34°F. Immature carrots (approximately 1/2" diameter, 30 g in weight, harvested eight weeks from planting) were observed to keep for approximately five weeks under these conditions. (The carrots retained good turgor, texture and were not infected.) However, within this five week period the carrots had accumulated large quantities of acetaldehyde (increased from one to eight ppm) and ethanol (increased from 30 to 1500 ppm) which are indicative of anaerobic respiration (Amerine, 1964; Cossins and Beevers, 1963). Evidently the polyethylene bags created a reduced oxygen tension due to the respired carbon dioxide. Apart from an increase to a few ppm of methanol the concentration of the other volatiles had not changed significantly at the end of the storage period.

With mature carrots (approximately 1-1/2" diameter, 150 g in weight) the storage period was observed to extend to at least three to four months. Under these conditions, a storage life of approximately five weeks for immature carrots and approximately four

months for mature carrots agrees well with the values reported by Lutz and Hardenburg (1968) for commercial storage. When samples of mature carrots in storage were examined at bi-weekly intervals it was not until the eighth week that the concentration of acetaldehyde and ethanol began to increase (up to 2 ppm and 1000 ppm, respectively). It was observed that although the carrots retained good turgor and taste the aroma changed towards the end of the storage life. A softer, "sweeter" aroma, which is believed due to acetaldehyde/ethanol developed. A similar but less pronounced trend occurred in the late season carrots in the soil. These carrots were not rotting, there was no infection or fermentation odor one would expect with very high concentrations of ethanol. Ethanol is only very weakly perceived at concentrations of 1000-2000 ppm (Bills and Keenan, 1968).

A parallel can be drawn between the "aging" of carrots in the soil, and in storage, in that accumulation of acetaldehyde and ethanol appear to be related to senescense. However, it must be remembered that in both of these instances these trends were believed to occur under anaerobic conditions.

An important conclusion from the maturity, variety and storage studies is that great care should be exercised in reciting the full history of carrots, and presumably of other fruit and vegetable products, used for flavor analysis. For many studies it is no longer

sufficient to merely claim the product was purchased at the local supermarket.

Effects of Processing on Carrot Volatile Constituents

This study was undertaken to determine the influence of processing, specifically canning and freeze drying, on the volatile composition of carrots. Both qualitative and quantitative changes were investigated in an attempt to determine which compounds were responsible for the characteristic raw and processed flavors (aromas).

Effects of Canning on Carrot Volatile Constituents

GLC-MS was used for analyzing the headspace volatiles present in aqueous extracts of the canned carrot. The same columns (TRIS and Carbowax 20 M) and conditions as used for separation of the "lower" and "higher" boiling compounds in the raw carrot were used in the analysis of the canned carrot. Figure 11 illustrates the separation of lower boiling volatiles on a TRIS column. The component identifications for the "lower boiling" compounds are listed in Table 13. The compounds identified support the findings of Self, Casey and Swain (1963) who surveyed the low boiling compounds present in vegetables which had been boiled for 30 min. The same "higher boiling" compounds found in the raw carrot were present in

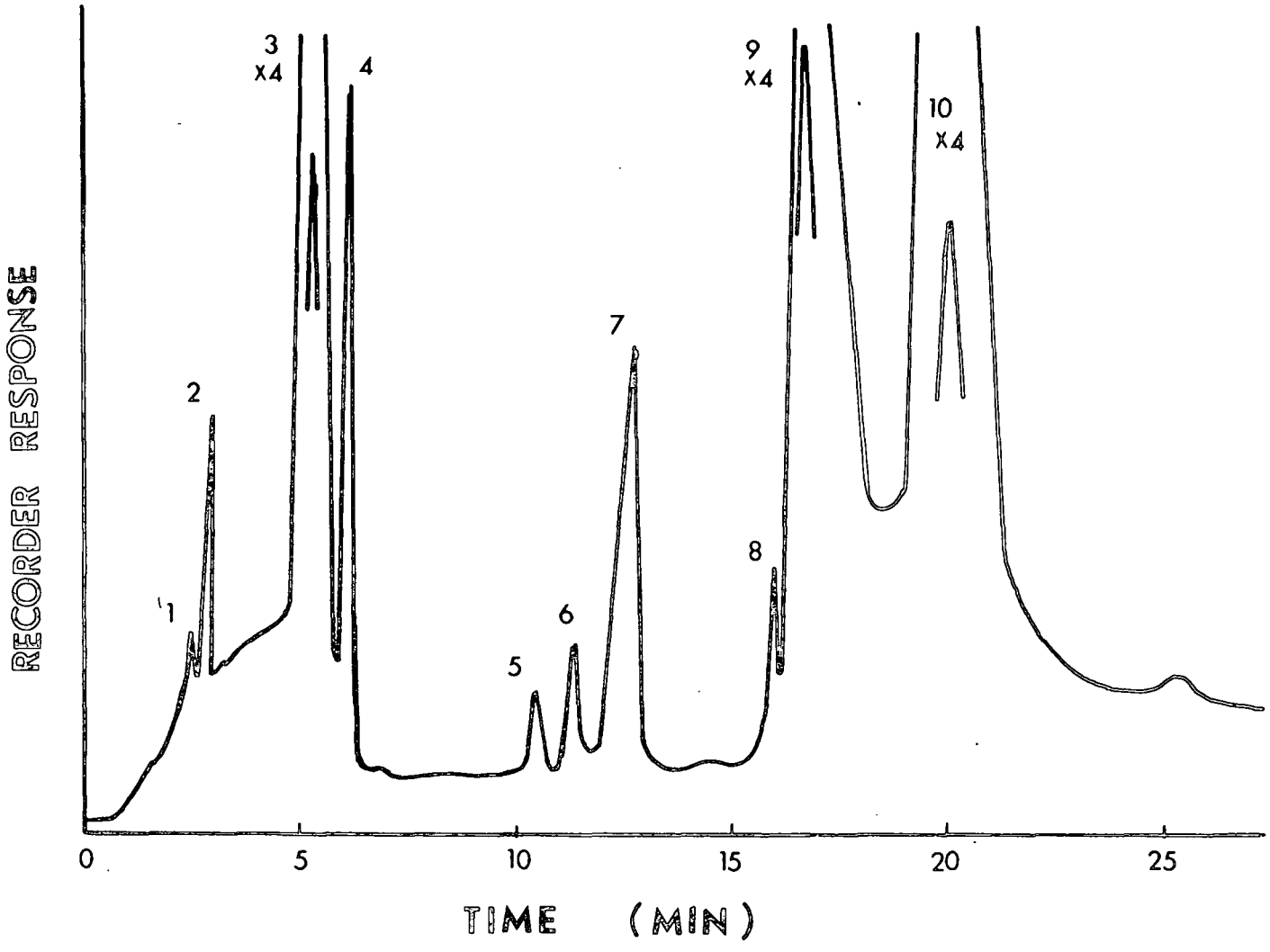

Figure 11. Analysis of the headspace volatiles present in an aqueous extract of canned carrots, using a TR1S column.

Table 13. GLC-MS identification of lower boiling compounds present in raw and canned carrots; chromatogram is shown in Figure 11.

Peak no.	Compound	tr/tr acetone	M.S. identification	M.S. reference	GLC confirmation	Aroma
1	Diethyl ether	0.14	+	Cornu & Massot	+	
2	Ethane thiol[a]	0.16	+	(1966)	+	+
3	Dimethyl sulfide[a]	0.30	+	ibid	+	+
4	Acetaldehyde	0.37	+	ibid	+	+
5	Unknown	0.60				
6	Propanal	0.67	+	ibid	+	
7	α-Pinene	0.75	+	ibid	+	+
8	Unknown	0.93				
9	Acetone	1.00	+	ibid	+	
	Methanol		+	ibid	+	
10	Ethanol	1.18	+	ibid	+	

[a]Compound only present in canned carrots.

the canned (Table 5) as well as significant amounts of three new incompletely identified aromatic compounds which appeared with retention times coinciding with peaks 14, 15 and 16 in Figure 5. On the basis of their mass spectra, all three compounds have a molecular weight of 132 and appear to be very closely related dimethyl substituted styrene isomers (ASTM, 1969). Peak 16 had the following mass spectrum: m/e 132 (molecular ion, 100%), 117 (85%), 91 (68%), 115 (36%), 92 (28%), 131 (25%), 116 (20%), 118 (13%) which agreed closely to the mass spectrum of α, p-dimethyl styrene, run on the same instrument. As the relative retention time of α, p-dimethyl styrene also coincided with that of peak 16, this compound is tentatively identified as α, p-dimethyl styrene. Peak number 15, based on the close agreement of relative retention time and mass spectrum [m/e 117 (100%), 132 (molecular ion, 87%), 115 (40%), 91 (40%), 131 (25%), 116 (24%), 133 (15%), 92 (8%)] is tentatively identified as 2, 6 dimethyl styrene. Peak number 14 appears to be an aryl α-dimethyl styrene compound on the basis of its mass spectrum (ASTM, 1969). The α, p-dimethyl styrene has also been reported in commercial distillates of black currants (Nursten and Williams, 1969).

Examination of the "lower boiling" volatiles in Table 14 indicates that canning has had the greatest effect on this fraction. Canning resulted in the formation and/or increase in concentration of seven of the eight headspace components identified. Ethanol was the

Table 14. Effects of processing on the "lower boiling" carrot volatile constituents.

Compound[a]	Raw Carrot	Canned Carrot	Freeze Dried Carrot
Ethane thiol	0.00	0.05	0.00
Dimethyl sulfide	0.00	1.55	Tr.[b]
Acetaldehyde	0.60	1.42	1.24
Propanal	Tr.	0.14	Tr.
Acetone	0.08	0.33	Tr.
Methanol	0.05	60.00	Tr.
Ethanol	50.00	45.00	7.36

[a] Concentration in ppm

[b] Trace

only compound whose concentration was relatively unaffected by the heat processing. Increases in acetaldehyde, propanal and acetone were not large whereas the increase in methanol from 0.05 ppm to in the order of 60 ppm was striking. The heat induction of a thiol (ethane thiol) and a sulfide (dimethyl sulfide) is of particular significance. These compounds were not found in the raw carrot. (Traces were formed by the headspace analysis method when a water bath temperature of 50°C was used, however, when the analysis was run at 25°C these compounds were not found to be present.) Actual increases were probably greater than those reported due to a probable loss of volatiles due to blanching of the carrots prior to peeling, slicing and canning. The Strecker degradation (Hodge, 1967) and oxidation of carotenoids (Swain, Fishwick and Land, 1964) can result in formation of acetaldehyde which can be detected by sensory analysis at concentrations of less than 1 ppm (Sheldon, 1968). Formation of propanal could arise from lipid auto-oxidation (Day, 1965; Schultz, Day and Sinnhuber, 1962) or from non-enzymatic browning (Hodge, 1967). The acetone is also a known end-product of non-enzymatic browning (Hodge, 1967). Formation of large amounts of methanol could arise from the hydrolysis of the methoxyl ester linkages in pectins. The finding of dimethyl sulfide correlates well with the reports of its formation in canned tomatoes (Nelson and Hoff, 1969), canned corn (Bills and Keenan, 1968) and in roasted filbert (Sheldon,

1969). Bills and Keenan (1968) confirmed the earlier report of Wong and Carson (1966) that dimethyl sulfide formation can result from the thermal degradation of an S-Methyl methionine sulfonium salt. The flavor threshold of dimethyl sulfide is only a few ppb (Bills and Keenan, 1968) and concentrations in the range of greater than 1 ppm as reported in this study are very significant. Ethane thiol has one of the lowest recorded thresholds (less than 0.1 parts per billion, Amerine, Pangborn and Roessler, 1965) and must be important in any detectable amounts. Acetone with an odor threshold of approximately 500 ppm (Wick, 1966) is unlikely to be important. While the threshold level of methanol is extremely high (the authors observations indicate methanol is not perceived by most people at concentrations of 1000 ppm) the large increase of this compound in canning could effect flavor by additive interactions as even mixtures of subthreshold levels of compounds have been found to initiate olfactory response (Guadagni et al., 1963).

Examination of the influence of canning on the "higher boiling" compounds in carrots (Table 15) indicates an approximate 50% reduction in total volatile content. This appears to result from a fairly uniform loss of the lower boiling monoterpenes and of the sesquiterpene caryophyllene. The concentration of the higher boiling sesquiterpenes γ and β-bisalsolene and carotol did not change significantly with canning. The loss of volatiles may be attributed to the

Table 15. Effects of processing on the "higher boiling" carrot volatile constituents.

Compound[a]	Raw Carrot	Canned Carrot	Freeze Dried Carrot
α-Pinene	0.09	0.10	0.04
Camphene	0.04	0.02	Tr.[b]
β-Pinene	0.05	0.05	Tr.
Sabinene	0.10	0.07	0.03
Myrcene	0.35	0.21	0.04
α-Phellandrene	Tr.	Tr.	Tr.
Limonene	0.46	0.22	0.06
γ-Terpinene	Tr.	Tr.	Tr.
p-Cymene	Tr.	0.45	Tr.
Terpinolene	6.05	2.50	0.46
Octanal	0.02	0.06	Tr.
α, p-Dimethyl styrene	0.00	(+)	0.00
Bornyl acetate	0.22	0.08	Tr.
Caryophyllene	10.00	5.00	1.95
Terpinene-4-ol	--	--	--
β-Bisabolene	1.18	0.87	0.60
γ-Bisabolene	5.55	3.50	2.15
Carotol	0.22	0.19	0.20
Myristicin	0.30	0.35	0.25
Total	26.00	14.00	6.00

[a] Concentration in ppm

[b] Trace

(+) Increase in concentration

blanching of the carrots prior to canning.

Compounds increasing with canning were p-cymene, octanal, 2-decenal and α, p-dimethyl styrene (and to a lesser extent the other two tentatively identified dimethyl substituted styrene compounds). Aldehydes such as octanal and 2-decenal could arise from the auto-oxidation of C_{18} unsaturated fatty acids (Schultz, Day and Sinnhuber, 1962). Although the increases in octanal and 2-decenal were not large these are potent compounds with very low thresholds of 0.7 and 0.3 parts per billion respectively (Buttery et al., 1968) and may be important in the cooked carrot aroma.

It is of interest to note the similarity in structure between p-cymene which increased on canning and α, p-dimethyl styrene (and the other dimethyl substituted styrene compounds) which was also formed on canning. The loss of two hydrogens from the p-cymene isopropyl group to form an isopropenyl group would convert p-cymene to p-methyl iso propenyl benzene (α, p-dimethyl styrene). As Wrolstad and Jennings (1965) found p-cymene can be formed from monoterpene hydrocarbons by isomerization and oxidation, it is possible this group of aromatic compounds may be formed by this mechanism during heat processing. They may also arise from dehydration of alcohols or degradation of carotenoids. It is felt that α, p-dimethyl styrene and related compounds contribute to stronger notes in canned carrot aromas. The peak tentatively identified as

α, p-dimethyl styrene had a strong, "weedy" (green) aromatic aroma. Whereas considerable amounts appeared in Imperator and Nantes varieties, only small amounts were present in the Chantenay and Oregon 4362.

The concentrations of individual terpenes found in the canned samples were still greater than the reported threshold levels (Buttery et al., 1968) indicating the changes responsible for the development of the characteristic cooked carrot aroma were probably not in this group of compounds.

Three other carrot varieties, Nantes, Chantenay and Oregon 4362 were also canned and the volatiles analyzed. In each instance apart from the previously discussed variation in α, p-dimethyl styrene the same trend in results as observed for the Imperator variety were obtained. These canned varieties were analyzed again after three months storage at room temperature (approximately 25^{o}C). There was no significant change in the results due to storage.

In summary, ethane thiol, dimethyl sulfide, acetaldehyde, aldehydes such as octanal, 2-decenal and possibly dimethyl substituted styrene compounds are considered important contributors to canned carrot flavor (aroma).

Effects of Freeze Drying on Carrot Volatile Constituents

The accelerated freeze dried carrots (Imperator variety)

although retaining a characteristic texture and taste only retained a moderate to weak raw carrot flavor (aroma). Examination of Tables 14 and 15 indicates freeze drying has resulted in a large loss of volatiles, in the order of a 75% reduction in the essential oil content.

Apart from the appearance of a trace amount of dimethyl sulfide there appears to be no characteristic changes induced by freeze drying in the "lower boiling" compounds (Table 14). The small amount of dimethyl sulfide may be regarded as arising from 'dry cooking' of the carrots. (An increase in low boiling compounds was noticed in stored, freeze dried material.) that had been exposed to the air. Increases in acetaldehyde and acetone in particular were noted. This agrees with the findings of Swain, Fishwick and Hand (1964) who hypothesized the compounds arise from carotene oxidation.

Examination of the "higher boiling" compounds (Table 15) indicates there is a lesser loss of the highest boiling compounds such as the bisabolenes, carotol and myristicin. The concentration of most compounds have been reduced to the level of their estimated thresholds. (The following threshold values, in ppm, were determined in water by Buttery et al. (1968): sabinene, 0.075; myrcene, 0.013; terpinolene, 0.2; bornyl acetate, 0.07; carotol, 0.008.) The reduction of most volatiles, particularly those such as sabinene, myrcene, terpinolene and carotol which are considered important in carrot flavor, to the viscinity of or below threshold concentration probably contributes to

the substantial loss of the characteristic raw carrot flavor (aroma) in the freeze dried carrot.

Perhaps the large loss in volatiles due to freeze drying is not surprising when one considers the high vacuum (approximately 100 microns) used in the freeze drying process. Undoubtedly freeze drying conditions could be found which would reduce this loss of volatiles. However, the results do serve to illustrate the extent to which volatiles may be lost in freeze drying.

One consideration arising from these results is the potential for trapping the volatiles removed by the freeze drying process. Such by products of fruits and vegetables could find use as a flavor concentrate on a commercial scale. Freeze drying would also appear to be an excellent, mild, non-destructive method for preparing flavor concentrates for laboratory analysis.

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APPENDIX

APPENDIX

RECOVERY OF VOLATILES BY REDUCED PRESSURE STEAM DISTILLATION

The aqueous distillate containing carrot volatiles was condensed in a series of cold traps as described in the Experimental section under Preparation of Freeze Dried Precursor Material. After thawing and combining the contents of the cold traps the aqueous distillate was extracted and concentrated.

Extraction

The aqueous distillate was extracted in a conventional continuous liquid-liquid extractor designed for solvents less dense than water. Three liters of distillate were saturated with sodium chloride and extracted continuously for 20 hours with approximately 250 ml of redistilled reagent grade ethyl ether or n-pentane.

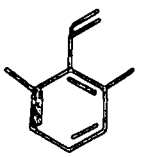
Concentration

After drying with excess reagent grade sodium sulfate, the solvent extracts were fractionally distilled to remove excess solvent using a 1x60 cm fractionation column packed with glass helices. A reflux ratio of 1:4 (collect to return) was controlled electronically with a control flow head. The extracts were concentrated to about

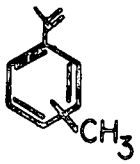
10 ml and stored. The extracts were further concentrated in chromatographic vials to as little as 10 μl under a nitrogen stream at 20-30°C.

Analysis

Examination of both ether and pentane extracts by GLC provided disappointing results as in both instances there was a very low recovery of carrot volatiles using this method. The possibility exists that the concentration to a very small volume with nitrogen at temperatures of about 30°C was too rapid, resulting in the loss of volatiles with solvent. As a carrot seed oil was available a carrot root oil was not essential to this study and the problem of obtaining one was not investigated further.



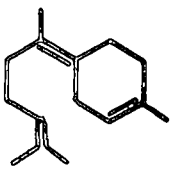
2, 6 dimethyl styrene



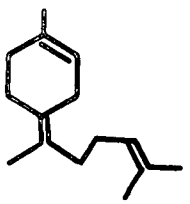
aryl α-dimethyl styrene

α, p-dimethyl styrene
(p-methyl isopropenyl benzene)

γ-bisabolone

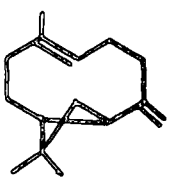


β-bisabolene

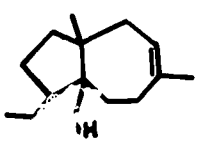


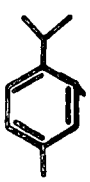
camphene

caryophyllene



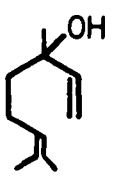
carotol



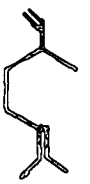


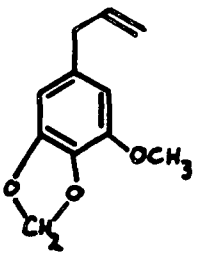
p-cymene

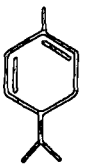
limonene



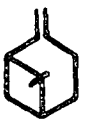
linalool

myrcene 

myristicin 

α-phellandrene 

α-pinene 

β-pinene 

sabinene 

γ-terpinene 

terpinolene 

terpinene-4-ol