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ANALYSIS OF ORGANOCHLORINE AND ORGANOPHOSPHATE PESTICIDE RESIDUES IN CABBAGE

A Thesis

Presented for the

Master of Science

Degree

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Adriance L. Prater

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ABSTRACT

Compounds present in cabbage (Cruciferae, family, genus Brassica, species oleracea, variety capitata) which interfere with pesticide analysis by an electron capture detector on a gas liquid chromatograph were investigated. The interfering compounds were found to be present throughout various segments of the cabbage head as well as in cabbage grown in different geographical areas under different growing condition. During storage of the cabbage up to 40 days at $2^{o}C$ prior to analysis, the interfering compounds decreased in concentration. After 16 days storage, some compounds disappeared while other compounds appeared; however, some interference remained up to 40 days of storage. Various treatments which did not remove the interference included: heat, and different clean-up methods by florisil columns, and acid-celite column, and an ion-exchange column. Mass spectrometry of interference from cabbage indicated the presence of N-octadecane, the existance of long chain hydrocarbon waxes, and the existance of alkyl substituted benzene rings. Mass spectrometry showed that volatiles which are a part of cabbage flavor (sulfur containing) or various pesticides were not the interfering compounds.

Recovery studies of organochlorine and organophosphate pesticides in which 2 to 16 ug were added to cabbage samples showed recoveries of 101 to 120% at 2 to 4 ug for α-BHC and 2 to 8 ug for lindane and β-BHC, and recoveries of 95 to 116% for p,p'-DDD and p,p'-DDT at 8 to 16 ug. Heptachlor, phorate, and methyl trithion showed low recoveries which increased to 67 to 76% recovery at 16 ug added. Other pesticides

(aldrin, p,p'-DDE, dieldrin, o,p'-DDD, o,p'-DDT, diazinon, and malathion) showed from 140-318% recovery, indicating more research is needed on the quantitation of these pesticides in cabbage by GLC-ECD using an internal standard.

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CHAPTER I

INTRODUCTION

Cabbage (Cruciferae family, genus Brassica, species oleracea, variety capitata) is a popular vegetable which had a U. S. production of 2.5 billion pounds in 1975. The plant is succeptable to a number of harmful insects and diseases, making the use of pesticides necessary for economical and adequate production. Although our agricultural economy and food supply could be jeopardized by the absence of chemicals, public concern has been aroused by the presence of pesticide residues in food. Furthermore, 85% of the edible bud or head of the cabbage plant is marketed in the fresh form with little or no further processing prior to consumption, making pesticide residue detection of major concern.

Organochlorine pesticide residue analysis of cabbage by present U. S. Food and Drug Administration Pesticide Analytical Methods Vol. I (FDA-PAM I) and A.O.A.C. methods is not adequate. The extraction and clean-up procedure do not remove compounds that interfere with the detection and quantitation of lindane and two of the isomers α-BHC and β-BHC, heptachlor, and aldrin by electron capture gas liquid chromatographic (GLC) analysis. Lindane may still be legally used to control pests on cabbage, and the possibility exists that cabbage may contain heptachlor and aldrin because of their long life in the environment and possible contamination from being used on non-food plants.

The interfering response is not identified as a chlorinated pesticide but is reported to be partly halogenated with a large sulfur response. Laboratory analysis of cabbage for pesticide detection on the GLC produces a cleaned-up extract which has the strong characteristic aroma of cabbage. Furthermore, the major naturally occurring volatile constituents of fresh cabbage have been identified as sulfur compounds. Reported sulfur and non-sulfur volatiles include: isothiocyanates, sulfides, disulfides, trisulfides, alcohols, aldehydes, and ketones. Various factors affect the volatile composition of cabbage, among which are: variety, maturity, cooking, and dehydration. Fresh cabbage also contains certain volatiles not present in sauerkraut.

Crops rich in sulfur, as cabbage, have been analyzed for the insecticide, Padan, following clean-up employing an ion exchange resin column to remove sulfur-containing compounds (Nishi et al., 1973). It has also been suggested that vegetable fractions may be further cleaned-up by passing them over a second florisil column or acid-celite column (U. S. FDA, 1971).

Thus this study was undertaken to locate, identify, and possibly remove the interfering compounds in cabbage, but still allow quantitative analysis of the previously mentioned organochlorine pesticides. Furthermore, it was believed necessary to analyze cabbage grown in different geographical areas to see if the interfering compounds were present. Also, since the much more toxic yet non-persistent organophosphate pesticides are replacing organochlorines in use, suitable multi-residue GLC detection methods for pesticide residue analysis of organophosphates should be evaluated.

CHAPTER II

LITERATURE REVIEW

I. GENERAL PESTICIDE ANALYSIS

Pesticide residue analysis usually consists of five steps as stated by Sherma (1979): (1) Sampling, (2) Extraction of residues from sample, (3) Purification of extract or "clean-up," (4) Identification and quantitation of residues in extract, and (5) Confirmation of presence and identity of residues. Variations of these stages are determined by the specific pesticide(s) and sample involved, and subsequently account for assorted analysis procedures. Mills et al. (1963) combined and refined previously reported procedures to provide a rapid method for quantitative analysis of organochlorine pesticides in non-fatty foods. This method of extraction and clean-up is the basis for analysis used by the Food and Drug Administration (FDA) in the Pesticide Analytical Manual, Vol. I (PAM I) (U.S. FDA, 1971). The Official Methods of Analysis of the A.O.A.C. (Horwitz, 1975) also contains this detailed description of extraction, clean-up, gas-liquid-chromatographic (GLC) and thin-layer chromatographic (TLC) procedures for multiple residue analysis of chlorinated and phosphated pesticides. Burke (1971) concluded in a review of the development of FDA's methodology as contained in PAM I, that this was the best available method for the determination of multiple organochlorine and certain organophosphate pesticide residues in food.

II. ORGANOCHLORINE AND ORGANOPHOSPHATE PESTICIDE RESIDUE ANALYSIS

Organochlorine pesticides are most often analyzed by gas chromatographic (GC) techniques utilizing electron capture (EC) detection at the, or less than the, nanogram amount. Oller and Cranmer (1975) concluded that while the electron capture detector has a narrow range of linearity, it is highly specific for chlorinated compounds. Aue (1975) supported the EC detector use for residues of chlorinated hydrocarbons but mentioned their ease of contamination from atmospheric oxygen or unclean samples.

The need for good methods of analysis for organophosphates is of great importance because of their increased usage due to curtailment of chlorinated pesticide use. Giuffrida (1964) and Nelson (1964) showed that the Mills et al. (1963) method of extraction could be applicable to certain organophosphate compounds, It was determined by Storherr et al. (1971) that the acetonitrile extraction procedure used by FDA for chlorinated hydrocarbon pesticides could be employed for organophosphorus pesticide residues. Smart (1977), representing a panel reporting to the Committee for Analytical Methods for Residues of Pesticides and Veterinary Products in Foodstuffs, reviewed a number of methodologies for determining residues of organophosphate pesticides in fruits and vegetables. The panel modified these procedures and the modified procedure produced mean recovery values between 80 to 100% of a range of pesticides in seven kinds of fruits and root or leafy vegetables. One of the procedures evaluated (Watts et al., 1969)

used a charcoal column clean-up followed by GLC analysis using a potassium chloride thermionic detertor.

Organophosphate pesticides are more readily detected by flame photometric, thermionic, and alkali flame ionization (AFI) detectors (Ford and Beroza, 1967; Watts and Storherr, 1968; Smart, 1977). Burchfield and Storrs (1975) suggested that a need for a multi-residue technique exists. They further demonstrated that while AFI detectors were developed specifically for phosphorus, the electron capture detector (ECD) yields responses to many organophosphates, although in most instances at lower sensitivity than for chlorinated compounds. Ford et al. (1975) also reported ECD use in the analysis of organophosphorus pesticides. Using a modification of an earlier procedure (Johnson, 1962),Hoelscher et al. (1968) successfully determined parathion and methyl parathion residues on cabbage utilizing an ECD.

III. RESIDUE CONFIRMATION

The identity of pesticide residues should always be confirmed by a different method than the initial analysis, since interpretation of results and quantitation are dependant on accuracy. Sherma (1979) stressed the importance of confirmatory evidence when the relatively nonspecific ECD is used and listed alternative methods including: TLC, selective GLC detectors, and the very powerful tool of mass spectrometry. Cochrane (1975) agreed that mass spectrometry was the ideal confirmatory technique; however, its use is often limited by availability, cost of instrumentation, and insufficient sample. Cochrane (1975), therefore, proposed chemical derivatization

techniques as a confirmatory tool.

Safe and Hutzinger (1973) and Ryan (1977) reviewed the use and various applications of mass spectrometry and concluded it was valuable in qualitative analysis of pesticides.

IV. CABBAGE PESTICIDE ANALYSIS

Pesticide use on cabbage is inevitable, due to its susceptability to numerous harmful insects and diseases (Ware and McCollum, 1975). In an investigation of electron capture gas chromatography for the analysis of multiple chlorinated pesticide residues in vegetables, Burke and Giuffrida (1964) experimented with cabbage. They sought to determine if an interfering response from the crop would exist during analysis for residues and if conditions provided a sensitivity of about 0.01 ppm for a compound. They used the Mills et al. (1963) extraction and clean-up procedures. The interference found by Burke and Giuffrida (1964) to be present in "A" or Fraction 1 of a cabbage extract showed an initial response covering the first five minutes of the chromatogram that could not be identified by electron capture GLC as a chlorinated pesticide. A representative chromatogram showing this interference is depicted in Figure 1. However, after concentration and microcoulometric GLC determination, the cabbage extract was determined to be at least partly halogenated showing a large sulfur response. The interference coincided with retention times of the pesticides lindane, heptachlor, aldrin, and heptachlor epoxide, in part. The possibility exists that at least some of the interferring compounds are part of the cabbage aroma since the extract smells like

Figure 1. Representative chromatogram showing interference in cabbage with pesticide analysis.(Oven temp. 205°C)

cabbage and several cabbage volatiles contain sulfur.

V. CABBAGE VOLATILES

The major naturally occuring volatile constituents of fresh cabbage have been identified as sulfur compounds (Dateo et al., 1957). Thioglucosides, sulfoxides and other non-volatile compounds found in cabbage are thought to be volatile precursors (Salunkhe and Do, 1976).

Clapp et al. (1957) reported estimated amounts of certain isothiocyanates which are enzymatically released from thioglucosides in fresh cabbage, and which are believed to be responsible for taste and odor. Using gas chromatography and mass spectrometry to examine fresh, dehydrated, and rehydrated cabbage volatiles isolated by vaccuum steam distillation and fractionation, Baily and co-workers (1961) reported twenty-four sulfur compounds including isothiocyanates, sulfides, disulfides, and trisulfides. Allyl isothiocyanate was largely present in fresh cabbage. They also observed the presence of non-sulfur components such as alcohols, aldehydes, and ketones. Additional volatile components of cabbage were identified by Buttery et al. (1976) and include 2, 4, 5-trithiahexane. These researchers noted that while cabbage, broccoli, and cauliflower had qualitatively generally the same volatiles, cabbage was the only one with appreciable amounts of but-3-enyl isothiocyanate, 2-phenylethyl isothiocyanate, and allyl isothiocyanate.

Salunkhe and Do (1976) postulate that a vegetable's aroma formation begins in the field where environmental factors such as climate, soil, macro- and micro-nutrients affect their photosynthesis

and characteristic aroma development. Furthermore, they stressed the importance of cultivar, maturity, and horticultural practices on aroma. MacLeod and MacLeod (1968) agreed that differences in volatiles exist between and among varieties as well as between young and old leaves. The heart of the cabbage has a stronger flavor due to a greater abundance of sulfur volatiles, whereas the older, outer leaves liberate greater amounts of aldehydes, ketones, and alcohols, although some sulfur volatiles do exist (MacLeod and MacLeod, 1970 b).

In another study (MacLeod and MacLeod, 1970 a) comparing fresh versus dehydrated cabbage volatiles the following was found concerning dehydrated samples: (1) allyl cyanide was greatly increased; (2) allyl isothiocyanate was virtually lost altogether; (3) more volatiles were present, as diethyl ether and trans-crotonitrile; and (4) fewer saturated alchols were present. This same study found conflicting results on changes in volatiles during storage of dehydrated cabbage. Although some samples showed only minor change over a three month period except for dimethyl sulfide reduction, other samples produced few volatiles other than acetone.

Lee et al. (1974) investigated the aroma of fermented cabbage and concluded that while certain volatiles of fresh cabbage were present, as allyl isothiocyanate and methyl trisulfide, many of the intense odor compounds previously reported were absent, as methyl isothiocyanate and methyl disulfide.

Cooking is another factor affecting cabbage volatiles. It has been shown that as cooking time increases: (1) saturated aldehydes increase; (2) sulfides increase; and (3) allyl isothiocyanate and allyl

cyanide increase up to 20 minutes but decrease thereafter due to exhaustion of their precursor, the thioglucoside sinigrin (MacLeod and MacLeod, 1970 b).

CHAPTER III

MATERIALS AND METHODS

I. GENERAL SAMPLE PREPARATION WITH GLC ANALYSIS

All cabbage was prepared and analyzed by the following standard procedure unless otherwise indicated. Samples of 100 g wedges were extracted and cleaned-up by the A.O.A.C. procedures as set forth in the FDA-PAM I General Methods for Nonfatty Foods of Organochlorine Residues (U.S. FDA, 1971). After extraction, clean-up, and elution from a Florisil Column, eluate A (6% ethyl ether in petroleum ether) and eluate B (15% ethyl ether in petroleum ether) for each sample were evaporated to near dryness on a steam bath at approximately 90°C and each brought back to 2 ml volume with pesticide grade n-pentane.

Extracts were analyzed on a Shimadzu Gas Liquid Chromatograph model GC-3BE equipped with an electron capture detector having a Ni 63 radioactive source and a 2.1 m x 2 mm inner diameter glass column packed with GP 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport (Supelco, Inc., Bellefonte, PA.). Conditions of the GLC were:

Nitrogen 20ml/min

Oven temperature .....205°C

Injection temperature .....215°C

Approximately 2 ul of each sample extract were analyzed at one time.

II. SOURCE OF INTERFERING COMPOUNDS IN CABBAGE HEAD

Three fresh, fairly well trimmed cabbage heads, having no more than seven wrapper leaves, were purchased from local grocery stores

for analysis. A Bush Brothers King Cole variety cabbage head was also analyzed. Initally, a 100 g wedge sample from each head was extracted to confirm the presence of the interference. Also, from each head were taken: a 100 g inner-most young leaves sample from around the heart and a 100 g outer-most wrapper leaves sample. Following analysis by the standard procedure, representative chromatograms of each portion of the head were obtained.

III. EFFECT OF GEOGRAPHICAL GROWING AREA ON INTERFERENCE

Cabbage was obtained from different geographical areas of the United States and analyzed according to the standard procedure. Six random samples of each source of cabbage were analyzed, and representative chromatograms of each geographically grown cabbage were obtained.

Cabbages of unknown varieties originating from Florida and California were randomly selected from local supermarkets during the winter months of 1979-80.

Cabbages of the variety, King Cole, were obtained from Bush Brothers and Company in the spring of 1979, and were grown in Dandridge, Tennessee, according to normal company procedures (Appendix Table A-1).

Cabbage plants of the same King Cole variety were supplied by Bush Brothers and Company and grown in the spring on a local Knox County garden with no previous pesticide use and without the aid of pesticides during the growing period.

IV. EFFECT OF STORAGE ON INTERFERENCE

Six large heads of cabbage, tentatively identified as the Little Rock variety (Maze, 1979), were received from Bush Brothers in the summer. A timed refrigerator storage study was initiated beginning with the arrival day of the cabbage to the laboratory. Three random 100 g wedges were taken from the six heads at 0, 8, 16, 32, and 40 days of storage under conditions of approximately $2^{o}C$ in brown paper bags. Samples were extracted and cleaned-up by the standard procedure. Representative chromatograms were obtained for each storage time.

V. REMOVAL OF INTERFERING COMPOUNDS

Attempts were made to remove compounds which interfered with detection and quantitation of 5 organochlorine pesticides (α-BHC, Lindane, β-BHC, Heptachlor, and Aldrin) and partially interfered with another (Heptachlor Epoxide). Methods were sought which would remove the compounds but not destroy or remove the pesticides. Among the methods tried were heating the cabbage and different clean-up procedures. Each method was tried twice on cabbage from which samples had been analyzed and was known to have the interfering compounds. Representative chromatograms of the cabbage with and without treatments were obtained.

Heating

A wedge of cabbage was brought to a boil in a beaker with 25 ml distilled water and heated for 30 minutes. Two 100 g samples were taken for analysis by the standard procedure.

Second Florisil Column

Following normal clean-up procedures, combined eluates A and B were evaporated to 10 ml and chromatography of this sample was repeated with a new Florisil column as directed in Section 211.15a of U. S. FDA (1971). The cabbage sample was again eluted with 6% ethyl ether in petroleum ether followed by 15% ethyl ether in petroleum ether.

Acid-Celite Column

Another clean-up procedure given in Section 211.15b of U. S. FDA (1971) was also tried. The cabbage eluate A from the Florisil Column was evaporated just to dryness, dissolved in 10 ml of carbon tetrachloride and transferred to an acid-celite column. The cabbage sample was eluted with 75 ml CCl_4. The eluate was concentrated to near dryness, brought to a volume of 2 ml in pesticide grade n-pentane and analyzed by GLC-ECD.

Ion-Exchange Column

The recommended method of Nishi *et al*. (1973) for the insecticide Padan ($C_7H_{16}CI\ N_3O_2S_2$) residue analysis in sulfur-rich crops was utilized in an attempt to clean-up cabbage extracts which supposedly contain sulfur compounds (Burke and Giuffride, 1964). The method involved a hydrichloric acid extraction followed by an ethyl ether extraction. Ion-exchange column chromatography, eluted with a 10% ammonium hydroxide solution cleaned up the extract. A final ethyl ether extract of the column eluate was taken prior to concentration and GLC analysis.

VI. IDENTIFICATION OF INTERFERING COMPOUNDS

A composited sample of the 6% ethyl in petroleum ether eluate of King Cole variety cabbage from Bush Brothers Company was prepared for analysis on a Model 5980 A Hewlett-Packard mass spectrometer attached to a Model 5710 A Hewlett-Packard gas chromatograph containing the same size and type column as described previously for pesticide analysis. The mass spectrometer also was equipped with a 5934 A data system. The sample represented 10 extractions of 100 g cabbage wedges (4 newly prepared extractions and 6 extractions which had been stored at -7°C for periods up to one month). Each extraction after being dried and dissolved in 2 ml pentane was checked for interfering compounds by GLC. All extractions were combined and concentrated on a steam bath at 90°C to a volume of about 0.5 ml. Ten ul of the sample were injected into the Hewlet-Packard gas chromatograph mass spectrometer for analysis. Gas chromatographic conditions were nitrogen flow, 20 ml/min; isothermal over temperature, 160°C; and injector temperature, 205°C. The mass spectrometer was set at a gain setting of 6 and had a 70 eV ionization potential.

Seventeen chemical compounds that are important volatiles in cabbage flavor (Dateo et al, 1957; Clapp et al., 1957; Bailey et al., 1961; Buttery et al., 1976; MacLeod and MacLeod, 1970a, 1970b) are listed in Table 1. Each of these pure compounds was diluted with n-pentane and approximately 200-300 ng were injected in the previously described GLC-ECD using the same column and conditions, except for an oven temperature of 160°C. Compounds with retention times that

TABLE 1

VOLATILE COMPOUNDS IN CABBAGE FLAVOR CHECKED AS CONTAMINANTS IN PESTICIDE ANALYSIS OF FRESH CABBAGE

Compound	Retention time matching cabbage contaminants
Crotononitrile[a]	
Ortho - Ethylphenyl Isothiocyanate[a]	
Hydrocinnamonitrile[a]	x
3 - Pentanone	
4 - Heptanone	
Hexyl alcohol	
Cis - 3 - Hexen - 1 - ol	x
Phenylacetaldehyde	x
trans, trans - 2, 4 - Decadienal	x
trans, trans - 2, 4 - Heptadienal	
trans - 2 - Hexenal	x
Allyl cyanide	x
Benzyl cyanide	x
Methyl disulfide	x
Allythiourea	
Sinigrin monohydrate	
Allyl isothiocyanate	x

[a]Compounds obtained from Alfred Bader Chemicals, Div of Aldrich Chem. Co. All others obtained from Aldrich Chemical Co., Milwaukee, WI.

corresponded to retention times of interfering compounds in various fresh cabbage samples under the same GLC-ECD conditions (Table 1) were then analyzed by GC-Mass Spectrometry. Approximately .25 ul of the pure compound was analyzed under conditions already described for cabbage sample analysis on the GC-Mass Spectrometer.

Organochlorine and organophosphate pesticide standards were analyzed under the same conditions except for a 205°C GLC oven temperature. Ten ul of an organochlorine pesticide standard mixture were injected (CP mixture ng/ul, Supelco, Inc., Belefont, PA). Contents in ng/ul were: α-BHC (0.025), β-BHC (0.100), Lindane (0.025), Heptachlor (0.025), Aldrin (0.050), Heptachlor-epoxide (0.080), p,p'DDE (0.100), Dieldrin (0.120), Endrin (0.200), o,p'-TDE (0.200), p,p'TDE (0.190), o,p'DDT (0.225), and p,p'-DDT (0.260). Ten ul of an organophosphate pesticide standard mixture were analyzed. The mixture contained each of the following in one ng/ml: Diazinon, Methyl Trithion, Malathion, Parathion, and Phorate (Thimet). The organophosphates were obtained from Supelco, Inc., Belefonte, PA.

VII. PESTICIDE RECOVERY IN CABBAGE

Cabbages of the King Cole variety, which were supplied by Bush Brothers and Company and stored under nitrogen at approximately -5°C for approximately 4 to 5 months, were analyzed and found to be free of pesticides. Samples of this cabbage were spiked with 2, 4, 8, or 16 ug/100 g sample of each of 13 organochlorine or of each of five organophosphate pesticides. All thirteen organochlorines, each at the same spiking level, were added simultaneously to a cabbage sample. All

five organophosphates were added in a like manner to another cabbage sample. Spiked samples were quantitatively analyzed for each pesticide by the standard procedure of extraction and clean-up; however samples spiked with organophosphates were eluted further from the florisil column by 50% ethyl ether in petroleum ether.

The previously described GLC-ECD was used with the same operating conditions that were used for analyzing cabbage. A Shimadzu Data Processor model Chromatopac-ElA was attached to the GLC for determination of peak area and quantitative calculations.

Organochlorine Pesticide Recovery

The organochlorine pesticides which were recovered from cabbage samples are listed in Table 2. They were obtained from Applied Science Labs., Inc., State College, PA. Five replications for recovery of each of the 13 pesticides at each added concentration were run. Elutions A (6% ethyl ether in petroleum ether) and B (15% ethyl ether in petroleum ether) were concentrated to dryness and brought back to a specified volumn with n-pentane, such that when eluates were combined for recovery analysis each pesticide was present at no more than 0.5 ng/ul.

An organochlorine pesticide standard mixture containing 13 pesticides was injected three different times into the GLC-ECD where each pesticide was at 1.0, 1.5, 2.0, 2.5, or 3.0 ng. The relative response factors (Rf) were calculated by the data processor programmed in Method 43 (unknown response factors) for each pesticide using heptachlor epoxide as the internal standard (see Appendix Table A-2).

TABLE 2

ORGANOCHLORINE AND ORGANOPHOSPHATE
PESTICIDE STANDARD MIXTURES

Number	Organochlorines	Number	Organophosphates
1	-BHC	1	Phorate
2	Lindane	2	Diazinon
3	-BHC	3	Malathion
4	Heptachlor	4	Parathion
5	Aldrin	5	Methyl Trithion
6	Heptachlor Epoxide		
7	p, p' - DDE		
8	Dieldrin		
9	o, p' - DDD		
10	Endrin		
11	o, p' - DDT		
12	p, p' - DDD		
13	p, p' - DDT		

The Rf was calculated according to the equation:

$$Rf = \frac{\frac{A_{IS}}{Wt._{IS}}}{\frac{A_P}{Wt._P}}$$

where A_{IS} = area of heptachlor epoxide peak

$Wt._{IS}$ = heptachlor epoxide weight in ug

A_P = area of pesticide peak

$Wt._P$ = pesticide weight in ug

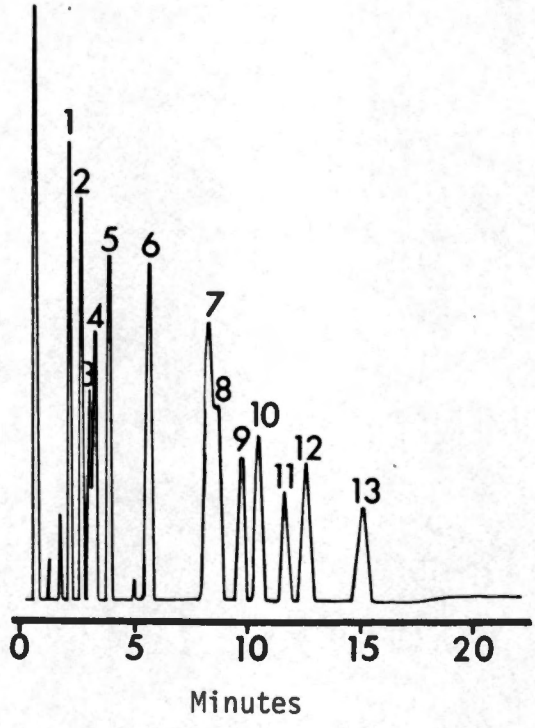
The linear range of the ECD for each pesticide was determined as that range where the Rf remained fairly constant (co efficient variation of $\pm$ 5% of a mean Rf in most cases).

Approximately 3 ul of each reconstituted combined eluate for each spiked sample[1] was injected into the GLC. Identification of each pesticide was made by comparing relative rentention times (RRT) with those of standards (Figure 2). The quantity of each pesticide in the spiked sample was obtained from the data processor programmed in Method 43 (known response factors) according to the equation:

$$\text{ug pesticide/100 g sample} = \frac{A_p \times R_f}{A_{IS}} \times \frac{Wt._{IS}}{Wt._S} \times 100$$

---

[1]The amount of each pesticide injected into the GLC for recovery studies was determined by adjusting volume of extract and volume injected into GLC such that the concentration of the pesticide, assuming 100% recovery, would have been within the linear concentration range of the ECD.



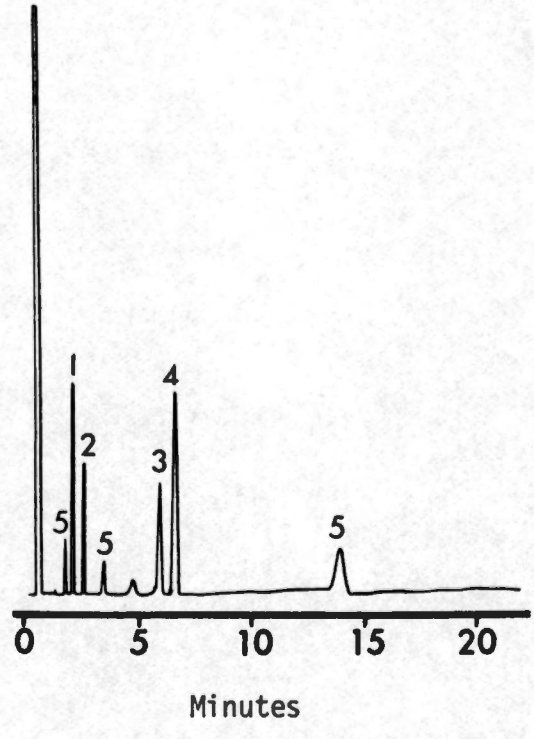


Figure 2. Representative chromatograms of (A) Organochlorine pesticide standard and (B) Organophosphate pesticide standard. (Oven temp. 205°C)

where

A_P = area of pesticide

R_f = response factor for pesticide

A_{IS} = area of heptachlor epoxide

$Wt._{IS}$ = heptachlor epoxide weight in ug

$Wt._S$ = sample weight in grams

Organophosphate Pesticide Recovery

The organophosphate pesticides which were recovered from cabbage samples are listed in Table 2. They were obtained from Supelco, Inc., Bellefonte, PA. Three replicates for recovery of each of the 5 pesticides at each added concentration were run. The three eluates 6%, 15% and 50% ethyl ether in petroleum ether, for each spiked sample were concentrated to dryness and then dissolved in n-pentane. The three eluates were combined in a final volume such that each pesticide was present at no more than lng/ul.

An organophosphate pesticide standard mixture containing 5 pesticides was injected three different times at 1.0, 1.5, 2.0, 2.5, or 3.0 ng into the GLC-ECD. The linear range of the ECD for each pesticide was determined. In that range the relative response factors were calculated, except parathion was used as the internal standard (See Appendix Table A-2).

Approximately 2 ul of each reconstituted combined eluate for each spiked sample was injected into the GLC. Pesticides were identified by comparison of relative retention time (RRT) to that of

standards (Figure 2). The quantity of each pesticide in the spiked samples was determined as described for organochlorine pesticides.

VIII. STATISTICAL ANALYSIS FOR RECOVERY OF PESTICIDES

The amounts recovered (ug) of each pesticide (dependent variable) were analyzed as a function of the amount of pesticide added to the cabbage sample prior to analysis (independent variable). The analysis of variance design as shown in Table 3 was run for each pesticide.

Various transformations of the dependent and independent variables were performed in an attempt to obtain a linear relationship between the two variables for each pesticide.

Table 3. Statistical analysis for pesticide recovery.

Source	Degrees freedom[a]	Degrees freedom[b]
Concentration	3	3
linear	1	1
quadritic	1	1
cubic	1	1
Rep (Conc)	16	8
Total	19	11

[a]organochlorine pesticides

[b]organophosphate pesticides

CHAPTER IV

RESULTS AND DISCUSSION

Typical interference from cabbage in the analysis of pesticides (U. S. FDA, 1971) is shown in Figure 1 on page 7. This interference which elutes from the GLC column from approximately 2 minutes to appromimately 5 minutes (ofter 6 minutes) affects the analysis of (1) α-BHC, (2) lindane, (3) β-BHC, (4) heptachlor, and (5) aldrin, and sometimes (6) heptachlor epoxide, all of which have retention times from 2 to 6 minutes (Figure 2). It has been reported that this interference consists of sulfur-containing compounds that are partly halogenated (Burke and Giuffrida, 1964).

I. SOURCE OF INTERFERING COMPOUNDS IN CABBAGE HEAD

Representative chromatograms of an extract from a cabbage wedge, inner-most leaves, and outer-most leaves are shown in Figure 3. The accepted sampling method for pesticide analysis in cabbage is to obtain a wedge of the head (U. S. FDA, 1971). Certain segments of the head were analyzed to observe if the interference from cabbage was specific to one area included in the wedge . The chromatograms indicated compounds responsible for the initial interference were present throughout the head. The chromatograms representing the extract from the segment of inner-most leaves was almost identical to that of the wedge. The chromatogram of the extract of outer-most wapper leaves also revealed the initial interference (2-5 min) and had additional inferfering compounds (Figure 3).

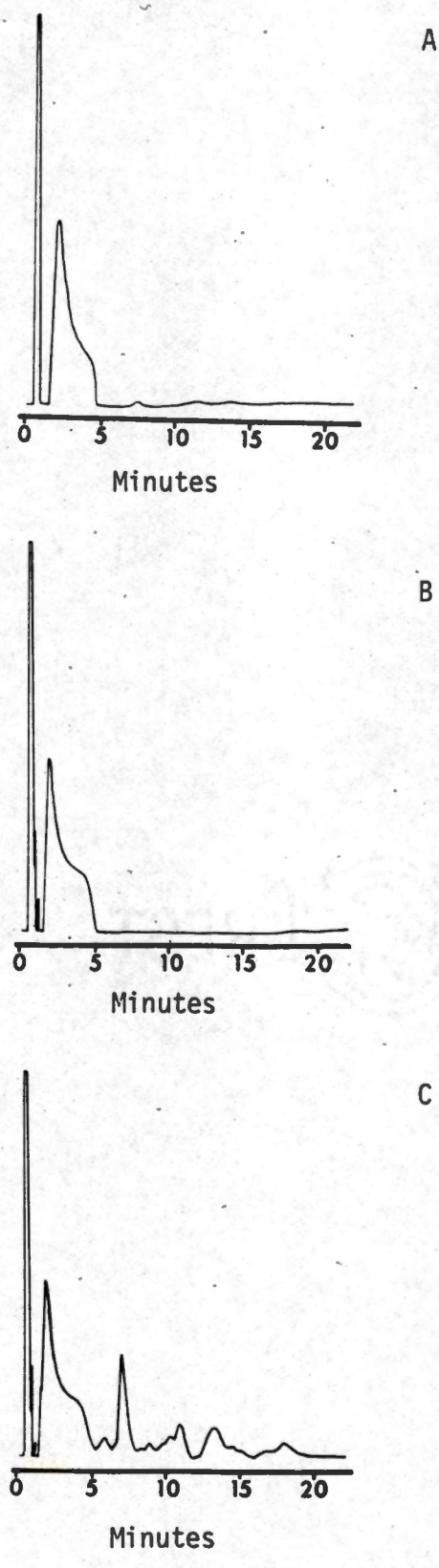


Figure 3. Representative chromatograms showing interference with pesticide analysis from cabbage (A) wedge, (B) inner leaves, and (C) outer leaves. (Oven temp. 205°C)

MacLeod and MacLeod (1968) indicated in a study of volatiles of cabbage that differences exist between young and old leaves. They further demonstrated (MacLeod and MacLeod, 1970b) that in general, sulfur volatiles were produced mainly from the young leaves around the heart, whereas the older, outer leaves liberated a large proportion of aldehydes, ketones, and alcohols. Outer leaves produced 8 times as much acetaldehyde as the inner leaves, but acetone was the most abundant of all volatiles. A whole cabbage head was found to form 36% sulfur volatiles compared with 46% for inner leaves and 15% for outer leaves.

Assuming that interference from cabbage is, in part, due to volatiles, the initial interference may be sulfur containing, with the additional interference in the outer leaves resulting from aldehydes, ketones, and alcohols.

II. EFFECT OF GEOGRAPHICAL GROWING AREA ON INTERFERENCE

Representative chromatograms of extracts from cabbage grown in different grographical areas are shown in Figure 4. All chromatograms of the samples showed the initial interference; however, the interference shown in the chromatograms of the California winter sample extract appeared to be much less concentrated than in the other samples. Both chromatograms (Figure 4-C and D) of the extracts from King Cole variety grown in Tennessee indicated a greater amount of interference than that from the two winter samples. Similarly, the interference in the chromatogram of the extract from the King Cole cabbage grown by Bush Brothers and Company in the spring of 1979 had a different shape and

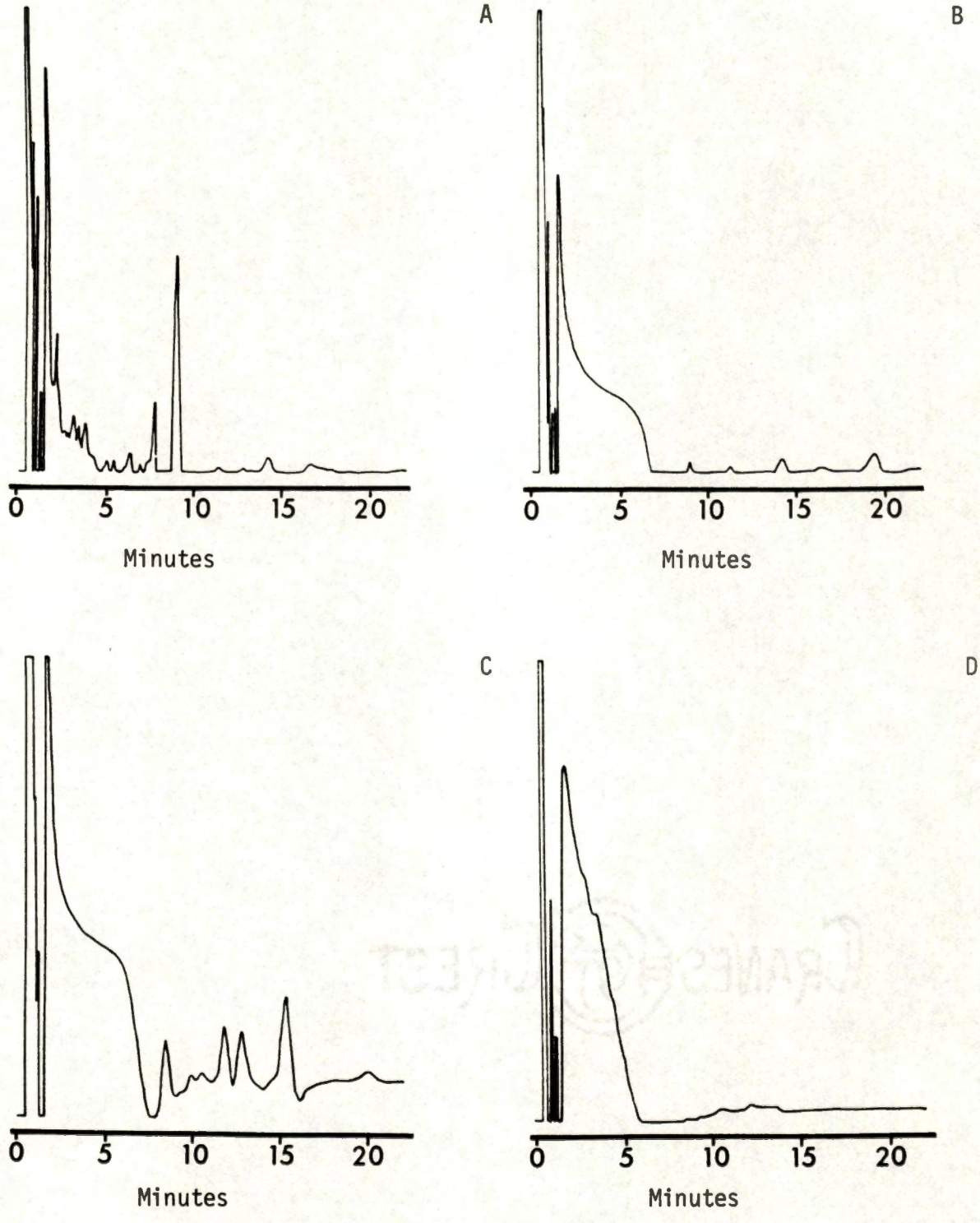


Figure 4. Representative chromatograms showing interference from cabbage with pesticide analysis from (A) California - winter, (B) Florida - winter, (C) Bush Brothers and Company - spring, and (D) local garden - spring samples. (Oven temp 205°C)

more peaks than did the chromatogram of extract from the same variety grown locally without pesticide application during the same period of time.

Salunkhe and Do (1976) attributed differences in vegetable aroma formation to environmental factors such as climate, soil and nutrients; and to other factors such as cultivar, maturity and horticultural practices. Various factors of cabbage grown in different geographical areas are discussed by Seelig (1969). This investigator reported, for example, that 100 to 200 pounds of nitrogen and 100 pounds of phosphorus pentoxide are added per acre in California, whereas a 6-8-8 (N-P-K) fertilizer is used in Florida. Also irrigation in the western states is by the furrow system, with the sprinkler system being used elsewhere. Too, different varieties grow best in different areas and in different seasons. King Cole is a hybrid variety which has had much success, especially in Florida (Seelig, 1969). Even though there were other numerous underlying variables in this particular study besides geographical growing area (ie. variety, nutrients, growing practices, growing season, maturity at harvest, etc.) the interference from cabbage with GLC-ECD analysis of pesticides existed throughout all samples. Slight differences in the shape and area of the interference shown by chromatograms of the extracts within the King Cole variety could have resulted not only from the pesticide use, irrigation, and fertilizer used by Bush Bros. and Co., but also from the increased maturity rate that these factors might have enhanced. The reasoning for a slightly less amount of interference from the California winter cabbage is at best speculative due to the many

encompassing variables. However, results were similar across all California samples. The length of time from harvest to actual analysis should also be considered, especially with the California cabbage samples.

III. EFFECT OF STORAGE ON INTERFERENCE

Representative chromatograms of extracts from King Cole variety cabbage analyzed for pesticides at 0 to 40 days of storage time at 2°C are shown in Figure 5. The initial interference (2-8 min. on the chromatograms) increased in area from 0 to 8 days storage. The area of this interference from cabbage remained fairly constant from 8 to 16 days storage. However, by 24 days storage (Fig. 5-D), there was a decreased area of the initial interference and several peaks appeared instead of one broad band as seen from samples stored up to 16 days. At 32 days storage, the same pattern of peaks existed that existed at 24 days, although some of the peaks showed increased area. At 40 days storage there was some change in the size and number of peaks in the interference pattern compared to that at the 32 day storage. Even though the initial interference was visibly decreased in area and separated into various peaks in chromatograms of extracts from cabbage stored longer than 16 days, peaks which interfered with analysis of pesticides were present in chromatograms of extracts from cabbage stored 40 days at approximately 2°C.

MacLeod and MacLeod (1970a) conducted an experiment on the flavor volatiles of fresh cabbage and dehydrated (air dried) cabbage stored up to 3 months in an oxygen present environment. Compared with fresh

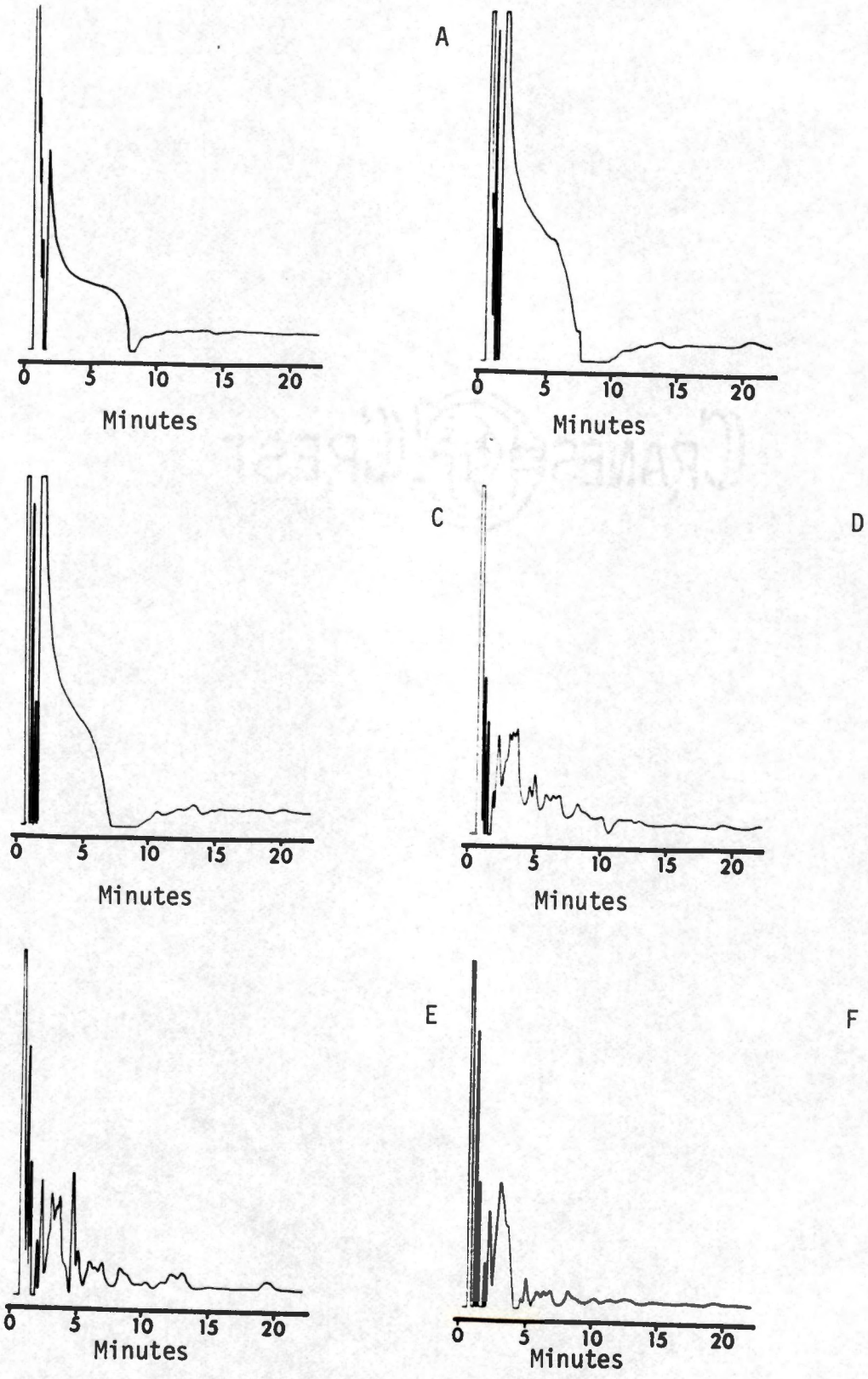


Figure 5. Representative chromatograms showing interference from cabbage samples stored for (A) 0 days, (B) 8 days, (C) 16 days, (D) 24 days, (E) 32 days, and (F) 40 days with pesticide analysis. (Oven temp. 205°C)

samples during this times, dehydrated samples produced: more volatiles overall, lower amounts of saturated alcohols (i.e. ethanol, n-hexanol, cis-hex-3-en-1-ol), increased amounts of formaldehyde and acetaldehyde, lower amounts of saturated aldehydes, dimethyl sulfide and allyl isothiocyanate, and increased amounts of nitrile compounds.

In view of this study the interference may initially be due to saturated alcohols, saturated aldehydes, dimethyl sulfide, and allyl isothiocyanate. However with storage beyond 16 days, these compounds in cabbage would be reduced in concentration while certain aldehydes and nitriles would actually increase.

IV. REMOVAL OF INTERFERING COMPOUNDS

Heating

Heating a cabbage wedge prior to pesticide analysis changed the initial interference (2-5 minutes) as shown by the chromatogram in Figure 6. Heating decreased the area of the interference due to compounds in the cabbage, possibly by causing reactions between compounds or by causing them to break down or rearrange into diffenent compounds. Heating apparently caused other compounds to be formed from precursors in the raw cabbage since peaks with retention times greater then 5 minutes were observed in the chromatogram of the heated sample extract (Fig. 6B).

In a study of cooked cabbage, MacLeod and MacLeod (1970b) found differences in volatiles and increased total volatile production with increased cooking time. Saturated aldehydes and sulfide increased continually with increased cooking time; whereas allyl isothiocyanate

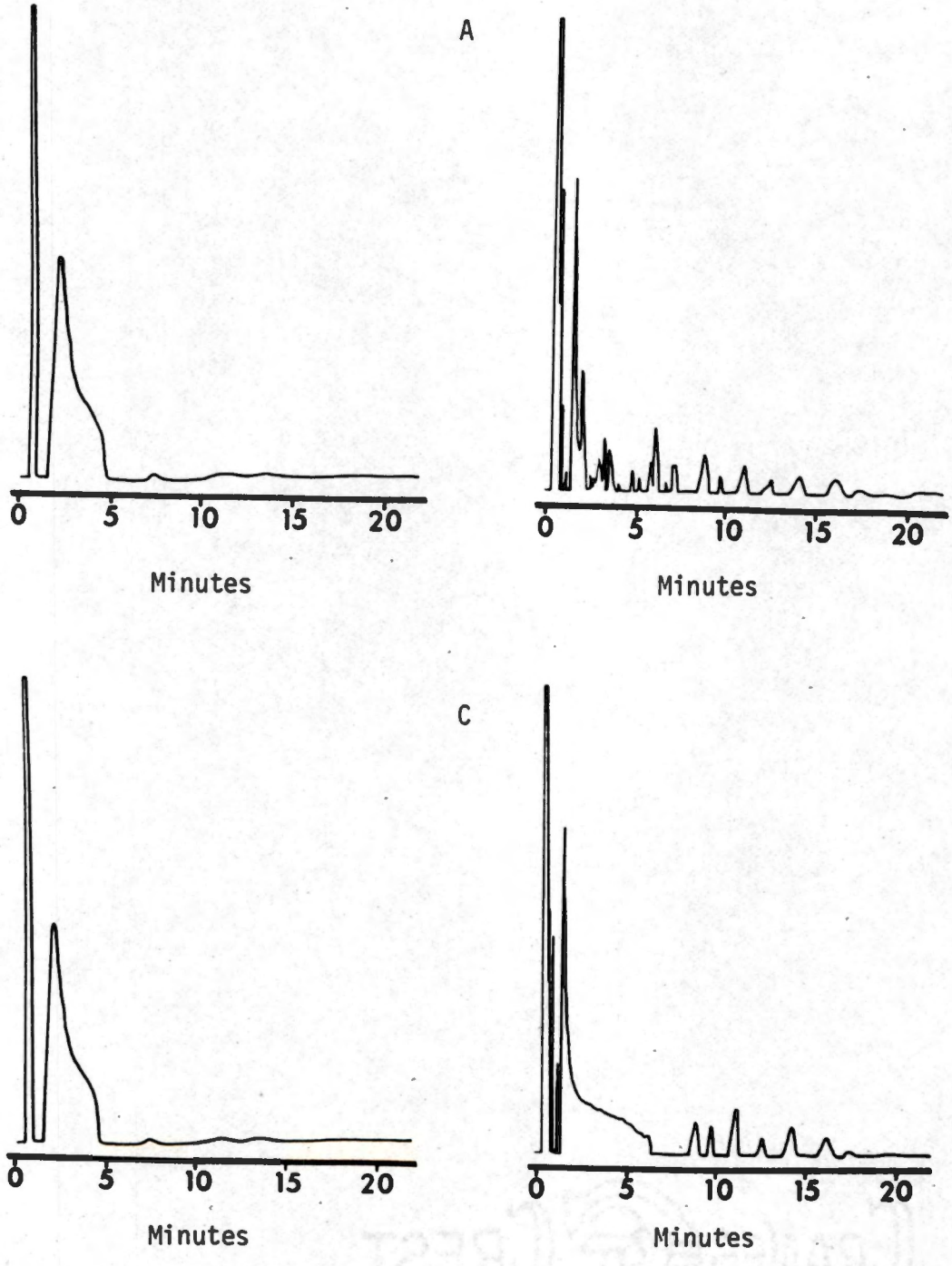


Figure 6. Representative chromatograms of interference with pesticide analysis from cabbage sample (A) without heating, (B) with heating, (C) with one florisil column clean-up procedure, and (D) with two florisil column clean-up procedures. (Oven temp. 205°C)

and allyl cyanide increased up to 20 minutes cooking time and decreased thereafter, because of the exhaustion of their precursor, the thioglucoside sinigrin. The compounds which are responsible for the initial interference from unheated cabbage (Figure 6-A) and which decreased in concentration after heating for 30 minutes (Figure 6-B) possibly could be, in part, allyl isothiocyanate and allyl cyanide rather than saturated aldehydes and sulfides, based upon the MacLeod and MacLeod (1970b) report. Peaks present after the first 5 minutes in the chromatogram of cooked cabbage (Figure 6-B) possibly could be aldehydes and sulfides, since they are not present in raw cabbage (Figure 6-A).

Second Florisil Column

Representative chromatograms of two samples from the same cabbage head, one which was cleaned-up by elution through one florisil column and another which was eluted through two florisil columns are presented in Figure 6. Clean-up by a second florisil column decreased the area of the interference slightly, but did not completely remove it. Also, additional peaks were found after the first 5 minutes of the chromatogram of the sample eluted through two florisil columns.

These results disagree with those found in the FDA-PAM 1 (U. S. FDA, 1971), where a second florisil column is suggested as a supplemental clean-up for vegetable extracts to remove compounds which interfere with pesticide recovery.

Acid Celite Column

The effect on the interference of a supplemental clean-up by an

acid-celite column is illustrated by representative chromatograms in Figure 7. The chromatogram of an acid-celite treated extract was very similar to that of the sample without additional clean-up except that a slight reduction in the area of the initial interference (2-5) min. was noted. Peaks throughout the remainder of the chromatogram essentially were unchanged.

These results did not reinforce the use of a supplemental acid-celite column as presented in the FDA-PAM (U. S. FDA, 1971), as an additional clean-up for extracts of products which contain compounds that interfere with pesticide analysis.

Ion-Exchange Column

GLC analysis of cabbage wedge extracts with and without an ion-exchange column clean-up are shown as representative chromatograms in Figure 7. The area of the initial interference (2-6 min) in the chromatogram of the sample without column clean-up was very large and it was not removed completely by ion-exchange clean-up. The column clean-up did remove compounds responsible for the peaks after 5 minutes in the chromatogram of the untreated sample.

The ion-exchange column method was proposed by Nishi *et al.* (1973) for analysis of certain pesticide residues in sulfur rich crops such as cabbage to remove sulfur containing compounds. Based upon the chromatograms of the cabbage extracts (Figure 7C and D), it would appear that only minimal amounts of sulfur compounds are present in the initial interference. These results disagree with the report of Burke and Giuffrida (1964) who reported a large sulfur

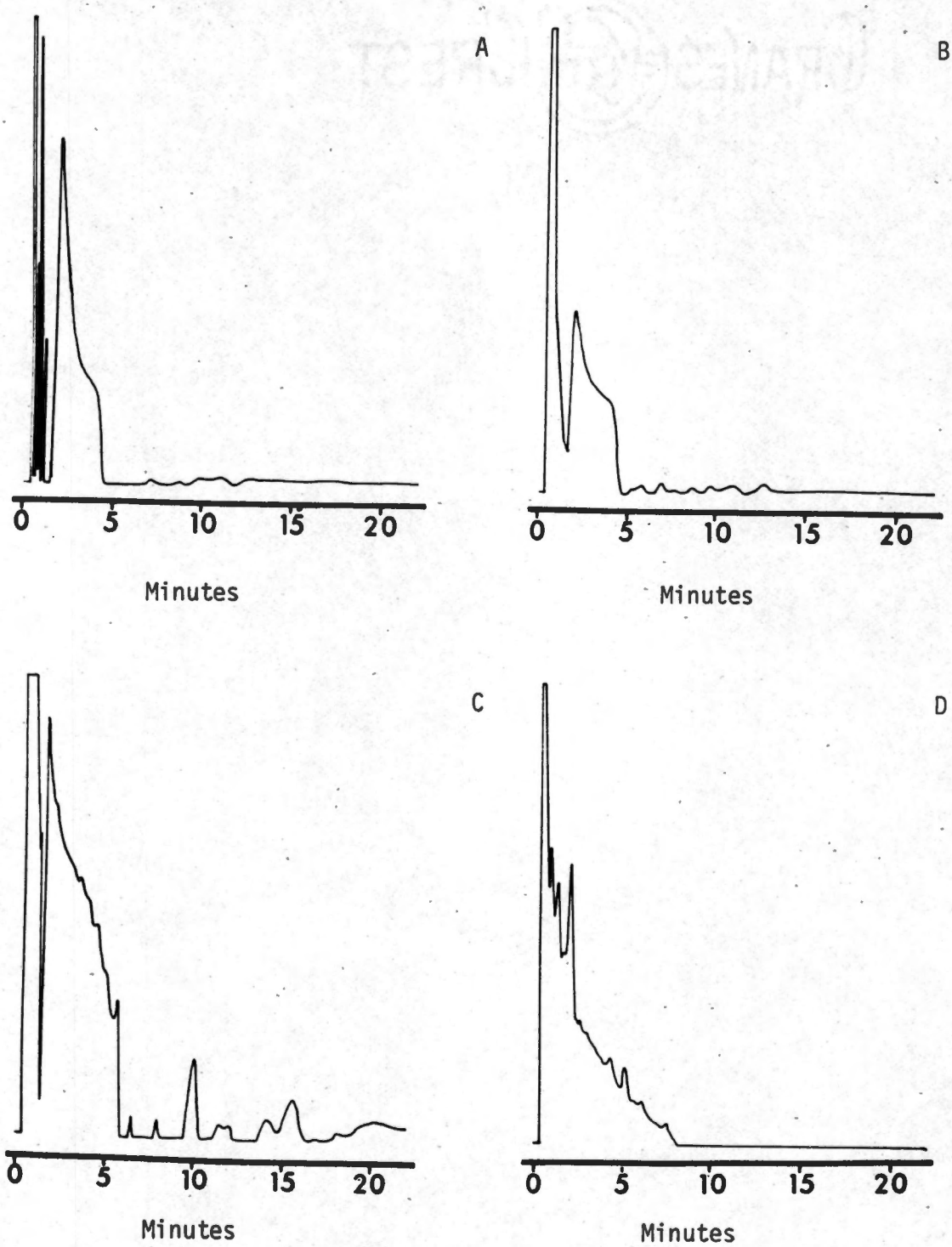


Figure 7. Representative chromatograms of interference with pesticide analysis from cabbage sample (A) without acid-celite clean-up, (B) with acid-celite clean-up, (C) without ion-exchange clean-up, and (D) with ion-exchange clean-up. (Oven temp 205°C)

response as well as halogens in the compounds causing initial interference from cabbage with pesticide analysis.

V. IDENTIFICATION OF INTERFERING COMPOUNDS WITH MASS SPECTROMETRY

Cabbage Analysis

A GLC-ECD chromatogram of the composited sample (ran with an oven temperature of 160°C) used for mass spectral analysis is shown in Figure 8-A and the corresponding GC-MS chromatogram in Figure 8-B. Peaks numbered the same in each chromatogram are due to the same compounds. Attempts were made at characterizing certain compounds by mass spectral analysis (Melton, 1980).

Since this chromatogram was obtained at an oven temperature of 160°C, peaks 1-5 (Figure 8) which had retention times less than 2 min. at 205°C on the same GLC column were not characterized or identified because the first pesticide to elute at 205°C had a retention time of 2 min. Furthermore, when pesticides were run at 160°C on the same column the first pesticide was eluted at a retention time of 4 min. However, GC-MS peak number 5 was identified and confirmed from mass spectral data as 2,6-Di-tert-butyl-4-methylphenol (BHT) as shown in the Appendix, Figures A-1 and A-2. BHT was an anomaly in the particular extract analyzed because its presence was not confirmed in other cabbage samples.

GC-MS peak number 6 (Figure 9) has an apparent molecular weight of 240. The loss of a m/e 77 from m/e 240 to m/e 163 which is a major peak in the mass spectrum corresponds to the loss of C_6H_5-.

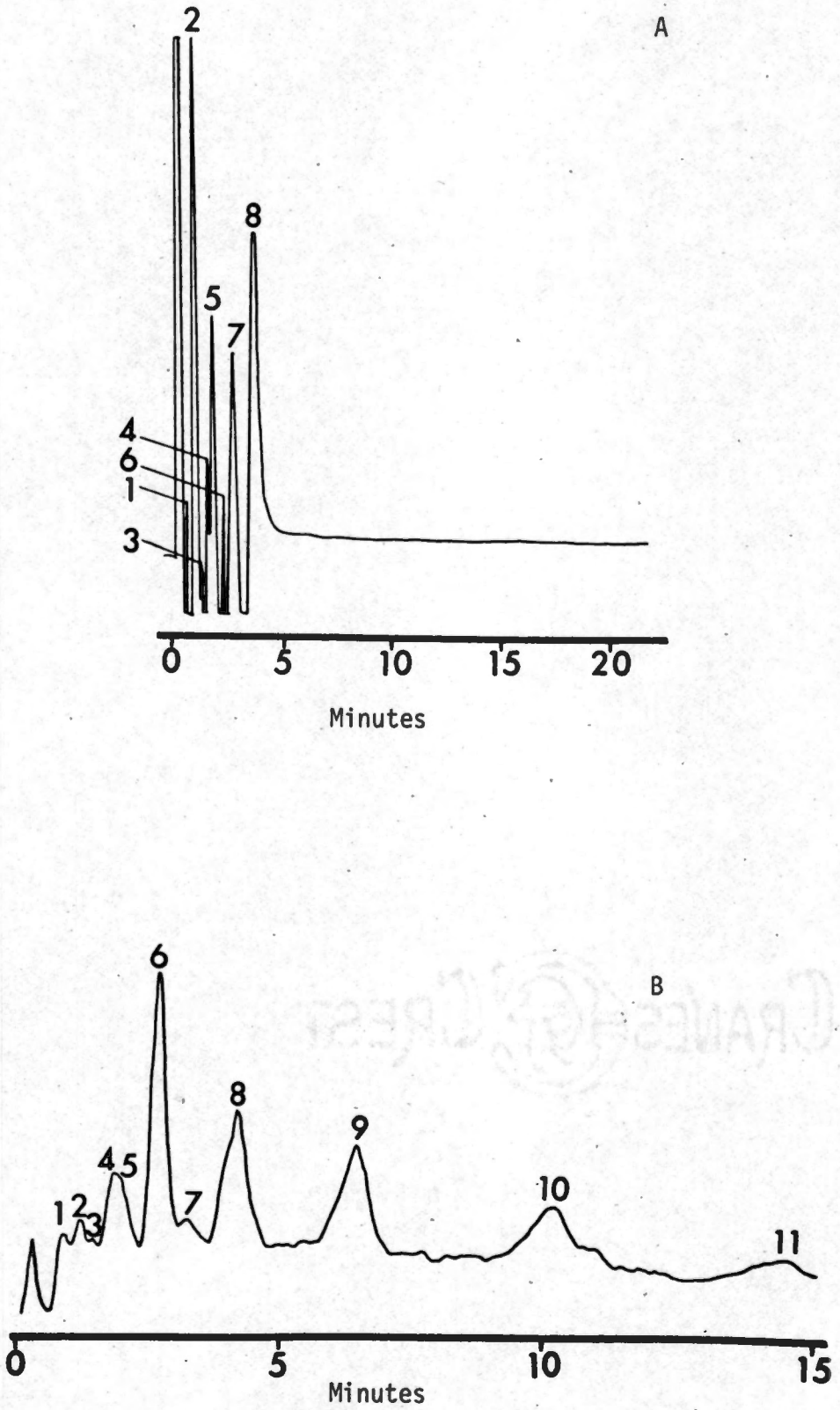


Figure 8. Representative chromatograms of corresponding compounds in composited cabbage sample with (A) GLC-ECD analysis and (B) GC-MS analysis. (Oven temp. 160°C)

MASS	ABUND	MASS	ABUND	MASS	ABUND
39	6.6	81	5.7	154	2.2
40	1.1	82	4.4	155	6.5
41	35.0	83	15.8		
42	6.8	85	54.6	163	37.7
43	65.2			164	3.3
44	1.5	91	91.9	165	1.6
		92	6.6	168	1.3
50	1.7	95	4.9	169	1.7
51	5.1	97	16.0	170	3.1
53	1.7	98	6.6		
54	2.0	99	13.1	182	1.6
55	33.4	103	3.9	183	3.7
56	19.6				
57	100.0	105	10.4	240	2.8
58	4.4	109	2.0	>PAUSE	
		112	7.0		
63	2.2	113	9.3		
65	10.3				
67	6.1	123	1.0		
68	2.3	125	2.9		
69	22.6	126	3.1		
70	17.2	127	5.4		
71	81.8				
72	10.3	137	.7		
		140	2.0		
77	9.3	141	3.6		
79	4.6				

Figure 9. GC-Mass spectrum of composited cabbage sample compound peak number 6.

The presence of the very strong peak at m/e 91 ($C_6H_5CH_2^+$) is indicative of an alkyl substituted benzene ring (Silverstein et al., 1974). The peak at m/e 105 ($C_6H_5CH_2CH_2^+$) may indicate branching at the α-carbon (Silverstein et al., 1974). The characteristic cluster of ions at m/e 77 ($C_6H_5^+$), 78 ($C_6H_6^+$), and 79 ($C_6H_7^+$) are due to α-cleavage and hydrogen rearrangement of monoalkyl benzene. M/e 65 ($C_5H_5^+$) results from elimination of a neutral acetylene molecule from $C_7H_7^+$ (m/e 91). The strong peak at m/e 163 is not easily identified from this spectrum. The best estimate at the present time indicates that it may be a $C_{10}H_{11}O_2^+$ ion which would indicate that the molecular formula for the parent peak at 240 is $C_{16}H_{16}O_2$. The peaks at m/e 57 and m/e 71 are most likely $C_4H_9^+$ and $C_5H_{11}^+$ respectively, due to isotopic abundance ratios at M+1 peaks. The peak at m/e 43 may be $CH_3\overset{O}{\overset{\|}{C}}^+$ based on isotopic abundance at M + 1, and the peak at m/e 85 may be $C_5H_9O^+$ based on the isotopic abundance at the M + 1 ion obtained from another mass spectrum of the same sample. Peaks at m/e 57 and 71 seem at variance with the other peak present in the spectrum. At best, GC-MS peak number 6 may be due to more than one compound which would make the identification from mass spectra very difficult.

The spectrum for GC-MS peak 7 (Figure 10) is probably incomplete as the parent peak is listed at m/e 253. The base peak or most intense fragment is at m/e 71. The mass spectrum shows a fragmentation pattern of 14 (CH_2) mass units with peaks at m/e 43, 57, 71, 85, 99, 113, and 127. Furthermore, the presence of the peak at m/e 91 suggests an alkyl substituted benzene ring (Silverstein et al., 1974).

MASS	ABUND	MASS	ABUND	MASS	ABUND	MASS	ABUND
39	4.4	78	1.7	110	7.8	173	1.7
41	39.4	79	8.1	111	26.3		
42	10.3	80	2.6	112	8.9	178	1.2
43	65.7	81	21.8	113	10.1	181	1.8
44	2.2	82	13.0	115	3.5	187	.7
		83	36.1	116	1.0		
51	1.0	84	19.6			193	2.1
53	4.5	85	60.5	118	1.1	197	2.8
54	5.2	86	4.4	119	6.3	198	2.2
55	53.2	89	.8	121	2.8		
56	25.7			123	9.0	204	1.0
57	98.9	91	18.0	124	3.5	205	1.5
58	5.7	92	3.0	127	9.5	207	.8
		93	5.0	128	4.3	209	.9
63	.7	94	3.1	129	3.2		
65	2.4	95	22.2			216	.9
66	1.6	96	13.5	133	4.6	221	1.1
67	18.0	97	42.0	134	1.9	222	.9
68	7.6	98	13.0	137	6.5	226	2.0
69	53.0	99	15.9	143	2.5		
70	30.5	100	1.3	144	1.1	235	.7
71	100.0	103	1.0	145	2.8	237	1.6
72	5.6					239	1.2
73	1.2	104	1.2	147	2.4		
		106	2.5	153	5.9	244	.7
76	.6	107	3.3	156	2.2	253	1.1
77	5.4	109	13.3			>Pause	

Figure 10. GC-Mass spectrum of composited cabbage sample compound peak number 7.

The characteristic cluster of ions at m/e 77 (C_6H_5+), 78 (C_6H_6+), and 79 (C_6H_7+) are due to α- clevage and hydrogen rearrangement of monoalkyl benzene. Further identification is not possible at this time.

GC-MS peak 8 has an apparent parent peak (M) at m/e 254. The base or most intense fragment is at m/e 57 (C_4H_9+). The mass spectrum (Figure 11) shows a fragmentation pattern characterized by clusters of peaks and the corresponding peaks of each cluster are 14 (CH_2) mass units apart. The largest peak cluster represents a C_NH_{2N+1} fragment (m/e 57) and the fragment intensities decrease in a smooth curve down to M- C_3H_7. The M - CH_3 and M - C_2H_5 peaks are missing. These characteristics are representative of saturated hydrocarbons (Silverstein et al., 1974). The smooth decrease in fragment intensities indicate a lack of branching according to these reporters. A molecular formula of $C_{18}H_{38}$ has the molecular weight of 254 and the spectrum of GC-MS peak 8 corresponds closely to the spectrum reported for n-octadecane (Middleditch, 1979).

GC-MS peak 9 has an apparent parent peak at m/e 268 (Figure 12). If the first loss from the parent peak is a loss of 40, which according to McLafferty (1973) is a loss of C_3H_7+ from aromatics, then the compound would contain a benzene ring. If the first loss from the parent peak is a loss of 57, then the characteristic shape of the rest of the spectrum indicates a long chain hydrocarbon with the formula $C_{19}H_{40}$. However in the cluster of peaks at m/e 43, 57, or 71, the isotopic abundance ratio of M + 1 to M does not indicate the presence of 3, 4, and 5 carbons for each respective m/e. The ions with m/e 43 could be CH_3CO, with m/e 57 could be $CH_3CH_2C = O$, and with m/e 71 could be

MASS	ABUND	MASS	ABUND
39	1.7	97	15.9
41	31.4	98	5.5
42	6.4	99	13.1
43	58.1		
44	1.2	109	3.2
		111	8.7
55	32.4	112	5.3
56	17.3	113	8.2
57	100.0		
58	3.3	125	4.0
		126	3.5
67	5.9	127	6.8
68	2.2		
69	22.0	140	1.8
70	16.1	141	3.3
71	75.2		
72	3.2	155	2.5
81	6.6	169	3.0
82	4.2		
83	16.0	182	1.6
84	9.7	183	2.3
85	51.3		
86	2.2	197	2.2
91	2.9	254	2.4
96	4.1	>Pause	

Figure 11. GC-Mass spectrum of composited cabbage sample compound peak number 8.

MASS	ABUND	MASS	ABUND	MASS	ABUND
41	35.0	97	20.4	228	2.1
42	6.4	98	6.8	229	1.5
43	66.9	99	13.4		
				268	2.5
54	1.6	109	4.4	>Pause	
55	37.4	111	11.1		
56	16.1	112	4.1		
57	100.0	113	7.4		
58	2.5				
		123	2.1		
67	7.5	125	5.3		
68	2.8	126	3.0		
69	28.4	127	4.7		
70	16.7				
71	77.4	137	1.8		
72	2.4	141	3.4		
79	1.9	155	2.5		
81	10.6				
82	5.5	165	1.8		
83	20.1	169	3.2		
84	10.0				
85	51.6	183	2.1		
91	2.1	196	1.5		
95	9.0				
96	5.6	211	2.2		

Figure 12. GC-Mass spectrum of composited cabbage sample compound peak number 9.

$CH_3CH_2CH_2C = O+$. The fact that there is not a strong peak at m/e 91 or 77 would indicate that the compound does not contain an aromatic ring (Silverstein et al., 1974) however, the presence of one or more oxygen molecules in the compound can not be ruled out from mass spectral data.

GC-MS peak 10 has an apparent parent peak at 282 (Figure 13). The same observations, except for parent peak minus m/e of 40 (M- 40), can be made for peak 10 that were made for GC-MS peak 9. What results in the apparent loss of 104 from the parent peak can not be even speculated at this time. One possibility is C_8H_8+ (McLafferty, 1973), but the lack of m/e 104 peak does not make this ion a logical choice. Isotopic abundance ratios of M + 1 to M for m/e peaks 43, 57, and 71 (from another mass spectrum of the same cabbage sample) indicate the possibility of oxygen being present in the compound.

GS-MS peak 11 has a m/e of 284 (Figure 14) which may be the parent molecule. The loss from parent m/e 284 of mass 27 to m/e 257 is most likely a C_2H_3+ loss and according to McLafferty (1973) is lost from $R\text{-}\overset{O}{\overset{\|}{C}}\text{-}O\text{-}C_2\text{-}H_5$. Peaks of m/e 41, 55, 69, 83, and 97, and 111 and 125 (from another mass spectrum of the same cabbage sample) are characteristic peaks for alkenes (C_NH_{2N}-1) (McLafferty, 1973). Peaks at m/e 63, 77, 91, 105, 119, and 133 are characteristic peaks for an alkyl substituted benzene ring (Silverstein et al., 1964). No other information is readily available from the mass spectrum of GC-MS peak 11.

The interference from cabbage during GLC pesticide analysis at 205°C is shown as a very broad GLC peak continuing up to and beyond 15 minutes when run at the lower 160°C (Figure 8-A). Therefore, mass

MASS	ABUND	MASS	ABUND
41	34.6	111	11.0
42	5.4	113	7.1
43	65.6		
		123	2.8
55	39.8	125	4.3
56	16.6	126	3.0
57	100.0	127	4.5
58	2.8		
		141	3.7
67	10.1		
69	29.5	151	1.9
70	15.7	155	2.4
71	75.1		
72	3.0	165	2.6
79	2.6	178	6.0
81	10.5	179	3.2
82	6.2	183	1.7
83	21.9		
84	9.5	282	2.6
85	49.9	>PAUSE	
95	11.2		
98	5.4		
99	11.2		
109	5.6		

Figure 13. GC-Mass spectrum of composited cabbage sample compound peak number 10.

MASS	ABUND	MASS	ABUND	MASS	ABUND
39	14.3	83	28.6	159	5.1
40	5.1	85	16.0		
41	100.0	88	8.0	161	17.7
42	18.9				
43	97.1	91	22.9	179	4.6
		92	4.0		
53	20.6	93	17.7	189	6.3
54	6.9	94	5.7	201	26.3
55	96.0	95	25.1		
56	20.6	96	7.4	257	8.6
57	65.7	97	21.1		
58	4.0			284	3.4
		105	18.9	>PAUSE	
65	8.6	106	4.6		
66	5.1	107	16.0		
67	52.6	108	6.3		
68	25.7	109	13.1		
69	50.3				
70	12.6	119	10.3		
71	30.3	121	8.6		
		125	4.6		
77	15.4	128	4.6		
78	3.4	131	3.4		
79	37.7				
80	5.7	133	6.9		
81	42.9	135	5.1		
82	12.0	145	5.1		

Figure 14. GC-Mass spectrum of composited cabbage sample compound peak number 11.

spectral data for two additional peaks (Figures 15 and 16), having retention times beyond 15 minutes were observed. Information for these peaks was obtained from other mass spectra of the same cabbage extract. Since GC-MS peak 11 had an apparent molecular weight of 284, and the highest m/e on the two spectra are 256 and 253, respectively, it is doubtful that the parent peak is given by each spectrum. However, both spectra indicate very complicated structures that could include saturated hydrocarbons, alkanes, alkenes, and a benzene ring.

Positive identification of GC-MS peak 8 as n-octadecane and the fact that most of these compounds are nonsaponifiable matter, following alkaline hydrolysis as set forth in FDA-PAM I (U.S. FDA, 1971) (Appendix Figure A-3) would indicate that the compounds from cabbage that interfere with organochlorine pesticide analysis are possibly plant waxes. Waxes are primarily esters of high-molecular fatty acids with high molecular alcohols (Delvaux and Bertrand, 1964). The fatty acids may be saturated and range from C_{16} to C_{26} with alcohols ranging from C_{12} to C_{31}.

Volatile Analysis

None of the mass spectra of the nine volatile compounds analyzed (Table 1, page 16) were similar to the spectra obtained for the chromatogram peaks representing the interference from cabbage with pesticide analysis. However, since this was not an exhaustive analysis of all volatiles reported in cabbage, it is possible that some of the interference could be a high molecular weight volatile or volatile

MASS	ABUND	MASS	ABUND	MASS	ABUND
39	5.0	91	5.0	192	3.3
41	56.7	93	3.6		
42	14.0	95	7.4	239	2.2
43	100.0	96	3.3		
44	3.3	97	10.2	256	3.0
		98	2.8	> PAUSE	
53	3.9	99	5.0		
54	3.9				
55	44.9	105	3.9		
56	16.8	109	4.7		
57	79.6	111	4.7		
58	3.0	112	2.2		
		113	3.0		
67	10.5				
68	4.4	121	2.2		
69	21.5	123	2.8		
70	8.8	125	1.9		
71	38.6	127	2.5		
72	2.2	129	1.9		
77	2.8	133	2.5		
79	5.8	141	2.2		
81	8.3				
82	4.1	169	1.7		
83	13.8				
84	5.0	189	2.2		
85	23.7	191	2.8		

Figure 15. GC-Mass spectrum of composited cabbage sample compound A beyond peak number 11.

MASS	ABUND	MASS	ABUND
39	5.3	84	6.6
40	3.3	85	22.5
41	62.7	87	4.1
42	14.8		
43	100.0	91	3.7
44	3.7	93	4.1
		95	9.0
53	4.5	97	10.7
54	5.7	98	4.5
55	54.1	99	5.3
56	18.0		
57	80.3	109	5.7
58	3.7	111	7.4
59	4.5	113	3.3
64	10.7	128	3.7
67	13.9		
68	5.7	141	3.3
69	28.3		
70	11.5	165	3.3
71	41.8	169	2.9
77	3.3	179	2.9
79	6.6		
81	11.9	239	2.5
82	5.3		
83	16.4	253	2.9
		>PAUSE	

Figure 16. GC-Mass spectrum of composited cabbage sample compound B beyond peak number 11.

precursor not investigated.

Also, since the sample for analysis consisted of ten extracts, some of which had been stored for several weeks, it is possible that there was a breakdown or rearrangement of structures of the interfering compounds, and an identification of the interference peaks by comparison of mass spectra with that of pure cabbage volatile compounds could not be made.

Pesticide Analysis

Mass spectra of organochlorine and organophosphate pesticide standards were obtained. Interfering compounds present in cabbage extracts used for pesticide analysis could not be identified as either 13 organochlorine or 5 organophosphate pesticides.

VI. PESTICIDE RECOVERY IN CABBAGE

Preliminary investigation revealed that organochlorine and organophosphate pesticide standards could not be combined and added to cabbage samples for recovery analysis due to various conflicts which existed between retention times (RT) of the two pesticide groups (Figure 2, Page 21). The organochlorine, α-BHC, with a RT of 2 minutes coincided with the RT for the organophosphate phorate. Both of these pesticides are reported to elute in 6% ethyl ether in petroleum ether (U. S. FDA, 1971). Likewise, the RT of the organochlorine lindane coincided with the RT of the organophosphate diazinon, however they are reported to elute in the 6% ethyl ether and 15% ethyl ether in petroleum ether elutions, respectively (U. S. FDA, 1971). Too, the

RT of the organochlorine heptachlor epoxide and the organophosphate malathion are very close to 5.5 minutes, although they are reported to elute in different solutions (U. S. FDA, 1971).

Preliminary investigations in this study further indicated that not all pesticides were completely eluted from the florisil column as had been reported (U. S. FDA, 1971). For example, the chosen organochlorine internal standard heptachlor epoxide was found in small amounts in the 15% ethyl ether in petroleum ether elution. Too. organophosphates were eluted from the florisil column with elutions greater than 15% and up to 50% ethyl ether in petroleum ether. Malathion is reported to be partially eluted at 50% (U.S. FDA, 1971). Therefore eluates of each pesticide group were combined for recovery analysis.

The linear concentration range of organochlorine pesticides is shown in Table 4. With the exception of pesticides 4 (heptachlor) and pesticide 13 (p,p'-DDT), the relative response factors (Rf) had a coefficient variation (C.V.) of less than 5% over the linear concentration range reported. The electron capture detector, although sensitive for organochlorine pesticide analysis, is noted for having a narrow linear range (Aue, 1975).

The linear concentration range of organophosphate pesticides is shown in Table 5. Pesticides 1 (phorate), 2 (diazinon), and 3 (malathion) had relative response factors with a C.V. greater than 5%, over the linear concentration range reported, again indicating the narrow linear range of the ECD (Aue, 1975).

Percent recoveries of organochlorine and organophosphate pesticides

Table 4 . Analysis of organochlorine pesticide standard response factors.

Pesticide[a]	Linear Conc Range (ng)	Mean R_f	S DEV.	C.V.
1	1.0-1.5	2.0595[c]	±0.0261	±1.27
2	1.0-2.0	1.8473[d]	±0.0666	±3.61
3	1.0-2.0	3.1983[d]	±0.0691	±2.16
4	1.0-2.0	2.9657[d]	±0.1839	±6.20
5	1.0-2.0	1.6290[d]	±0.0483	±2.96
6	1.0-2.0	1.0000[d]	IS[b]	IS[b]
7	1.0-2.0	0.9671[d]	±0.0273	±2.82
8	1.5-2.0	2.1485[c]	±0.0813	±3.78
9	1.5-2.0	1.3185[c]	±0.1218	±1.66
10	1.0-2.0	1.4470[d]	±0.0269	±1.86
11	1.0-1.5	1.3640[c]	±0.0184	±1.35
12	1.0-1.5	1.4460[c]	±0.0424	±2.93
13	1.0-2.0	0.8264[d]	±0.0927	±11.22

[a]Pesticide numbers correspond to organochlorine pesticides in Table 2

[b]IS = Internal standard

[c]N = 6

[d]N = 9

Table 5. Analysis of organophosphate pesticide standard response factors.

Pesticide[a]	Linear Conc Range (ng)	Mean R_f	S. DEV.	C.V.
1	2.0-2.5	1.705[c]	±0.2192	±12.86
2	2.0-2.5	1.600[c]	±0.1414	±8.84
3	1.5-2.0	3.315[c]	±0.5728	±17.28
4	1.5-2.5	1.000[d]	IS[b]	IS[b]
5	1.5-2.5	1,6379[d]	±0.0544	±3.3213

[a]Pesticide numbers correspond to organophosphate pesticides in Table 2

[b]IS = internal standard

[c]N = 6

[d]N = 9

from cabbage with added concentrations of 2 to 16 ug are shown in Tables 6 and 7. The sum of squares for analysis of variance for organochlorine and organophosphate pesticides are shown in Tables A-3 and A-4. Higher terms (quadratic and cubic) of a model for amount recovered as a function of amount added of each pesticide were significant for several pesticides. However, since 98% or greater of the total variation (sum of squares) due to U (added concentration effect) was associated with the linear term, no higher order terms were included in the respective model for each pesticide shown in Tables 6 and 7 (Sanders, 1980). The model included the transformation of amount recovered and amount added to $\log_e$ terms.

In the recovery of organochlorine pesticides from cabbage, pesticides 7 (p,p'-DDE) and 8 (dieldrin) gave one GLC-ECD chromatogram peak and were therefore analyzed together (Table 6). It was also found that pesticide 10 (endrin) did not appear as a peak. However, the high percent recoveries for pesticide 9 (p,p'-DDD) and pesticide 11 (o,p'-DDT) would appear to indicate a transformation of endrin to compounds which elute with these two pesticides. All percent recoveries were generally high (at or above 100%) with the exception of pesticide 4(heptachlor). Pesticides 1, 2, 3, and 4 showed increased percent recoveries with increased amounts added, whereas pesticides 5, 7 and 8, 9, 11, 12, and 13 showed the reverse.

In the recovery of organophosphate pesticides from cabbage, pesticide 5 (methyl trithion) appeared as more than one chromatogram peak and amounts of two peaks were combined for recovery. Recovery of pesticides 1, 3, and 5 increased with an increased addition of

Table 6. Analysis of organochlorine pesticide recovery in cabbage.

Pesticide[a]	Linear Equation for Amount recovered	Amount added			
		2 ug	4 ug	8 ug	16 ug
		% Recovered			
1	$Log_e A^b = 1.1965\ U^c - 0.1182$	101.82	116.67	133.70	153.21
2	$Log_e A = 1.0684\ U + 0.0221$	107.20	112.40	117.86	123.58
3	$Log_e A = 1.0139\ U + 0.1228$	114.16	115.27	116.38	117.51
4	$Log_e A = 1.8391\ U - 2.7205$	11.79	21.07	37.70	67.43
5	$Log_e A = 0.9709\ U + 0.4641$	155.88	152.77	149.72	146.73
7 & 8	$Log_e A = 0.8028\ U + 1.1663$	279.99	244.23	213.02	185.81
9	$Log_e A = 0.9532\ U + 0.4696$	154.83	149.88	145.10	140.47
11	$Log_e A = 0.9011\ U + 0.8026$	208.35	194.55	181.66	169.62
12	$Log_e A = 0.8336\ U + 0.4384$	138.09	123.09	109.68	97.73
13	$Log_e A = 0.7150\ U + 0.7458$	173.02	142.01	116.55	95.66

[a]Pesticide numbers correspond to organochlorine pesticides in Table 2.

[b]A = ug of pesticide recovered.

[c]U = Log_e ug of pesticide added to cabbage.

Table 7. Analysis of organophosphate pesticide recovery in cabbage.

Pesticide	Linear Equation for amount recovered	Amount added			
		2 ug	4 ug	8 ug	16 ug
		% Recovered			
1	$Log_e A^b = 1.0867\ U^c - 0.5218$	63.02	66.92	71.07	75.47
2	$Log_e A = 0.5311\ U + 0.7764$	157.05	113.47	81.98	59.23
3	$Log_e A = 1.1022\ U + 0.9449$	276.14	296.41	318.17	341.53
5	$Log_e A = 1.2042\ U - 0.9037$	46.67	53.76	61.94	71.35

[a]Pesticide number corresponds to organophosphate pesticide in Table 2.

[b]A = ug of pesticide recovered.

[c]U = Log_e ug pesticide added to sample.

pesticide to the cabbage, whereas pesticide 2 (diazinon) showed the reverse.

CHAPTER V

SUMMARY

Compounds present in cabbage which interfere with pesticide analysis by an electron capture detector on a gas liquid chromatograph were investigated.

The interfering compounds were found to be present throughout various segments of the cabbage head including outer and inner leaves. The interfering compounds were present in cabbage grown in different geographical areas under different growing condition. However California winter samples appeared to have a lower amount of interfering compounds than other Florida or Tennessee samples.

The initial interference as shown by a GLC-ECD chromatogram of cabbage extract changed with storage up to 40 days at $2^{o}C$ of the cabbage. There was an increase in interference concentration from 0 to 8 days with no additional change up to 16 days. Thereafter, there was a decrease in concentration and some compounds disappeared while certain others appeared. Some interference remained with up to 40 days of storage.

Attempts were made to remove the interference. Although heating the cabbage caused some changes and decreased concentration of the interference, it was not removed. Different clean-up methods by florisil columns, and acid-celite column, and an ion-exchange column did not greatly effect or completely remove the interference.

Mass spectrometry of the interference in cabbage during pesticide analysis indicated the presence of N-octadecane, the existence of long chain hydrocarbon waxes, and the existance of alkyl

substituted benzene rings. Certain volatiles normally present in cabbage, 13 organochlorine pesticides, and 5 organophosphate pesticides did not have mass spectra similar to that of peaks which interferred with pesticide analysis and which were in the chromatogram of the cabbage extract.

Recovery studies of organochlorine and organophosphate pesticides in which 2 to 16 ug were added to cabbage samples showed recoveries of 101 to 120% at 2 to 4 ug for α-BHC and 2 to 8 ug for lindane and β-BHC, and recoveries of 95 to 116% for p,p'-DDD and p,p'-DDT at 8 to 16 ug. Heptachlor, phorate, and methyl trithion showed low recoveries which increased up to 67 and 76% recovery at 16 ug added. Other pesticides (aldrin, p,p'-DDE, dieldrin, o,p'-DDD, o,p'-DDT, diazinon, and malathion) showed apparent recoveries of 140 to 318% recovery. Since there were often large recoveries for certain pesticides, along with the appearance of p,p'-DDE and dieldrin as one GLC-ECD chromatogram peak and the absence of an endrin peak, indications point to the need for more research on the quantitation of these pesticides in cabbage by GLC-ECD using the internal standard method.

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APPENDIX

Table A-1. Bush Brothers and Company Cabbage Production (Harrison, 1980)

1. Plants with an unknown pesticide history are purchased from Florida in the early spring.

2. Land is contracted out by the company in the Dandridge, Tennessee area for growing the plants.

3. As the weather temperature increases, pesticide application is begun, and the plants are sprayed heavily throughout the growth period.

4. Plants are irrigated and fertilized.

Table A-2. Process of choosing internal standard.

1. Preliminary work to find an internal standard that was suitable for organochlorine pesticide analysis was unsuccessful after investigating several compounds.

2. Those compounds had retention times (RT) that were the same or close to those of organochlorines, or had retention times which were too ling or short to be suitable for pesticide analysis.

3. Investigators have used pesticides as internal standards for analyzing other pesticides (Zwieg, 1972).

4. Heptachlor epoxide was chosen because the interference in cabbage did not affect the GLC heptachlor epoxide peak, the RT was centrally placed among those of organochlorines, and it had a sharp peak. (Selection of parathion as organophosphate internal standard followed same reasoning.)

Table A-3. Analysis of variance sum of squares of organochlorine pesticide[a] recovery from cabbage.

Source	df	Pesticides 1	2	3	4	5	7 & 8	9	11	12	13
U[b]	3	21.798	20.533	21.589	8.153	22.531	15.097	17.268	17.298	17.883	17.548
linear	1	21.370[c]	20.293[c]	21.379[c]	8.014[c]	22.242[c]	15.048[c]	17.241[c]	17.120[c]	17.706[c]	17.392[c]
quad.	1	0.392[c]	0.217[d]	0.155[e]	0.126	0.267[d]	0.006	0.005	0.178[e]	0.078[e]	0.057
cubic	1	0.036	0.023	0.055	0.013	0.022	0.043[e]	0.022	0.000	0.099[e]	0.099
Rep (C)	16	0.166	0.169	0.266	3.386	0.359	0.140	0.171	0.499	0.249	0.424

[a]Pesticide numbers correspond to pesticides in Table 2.

[b]U = log of concentration added.

[c]Significant at P<.0001 level.

[d]Significant at P<.01 level.

[e]Significant at P<.05 level.

Table A-4. Analysis of variance sum of squares of organophosphate pesticide[a] recovery from cabbage.

Source	df	Pesticides 1	2	3	5
U[b]	3	13.243	16.831	9.873	10.192
linear	1	13.058[c]	16.030[c]	9.840[c]	9.945[c]
quad.	1	0.166[e]	0.745[d]	0.004	0.246
cubic	1	0.019	0.053	0.029	0.001
Rep (C)	8	0.140	0.289	0.125	0.412

[a]Pesticide numbers correspond to pesticides in Table 2, Page 19.

[b]U = log of concentration added.

[c]Significant at $P<.0001$ level.

[d]Significant at $p<.01$ level.

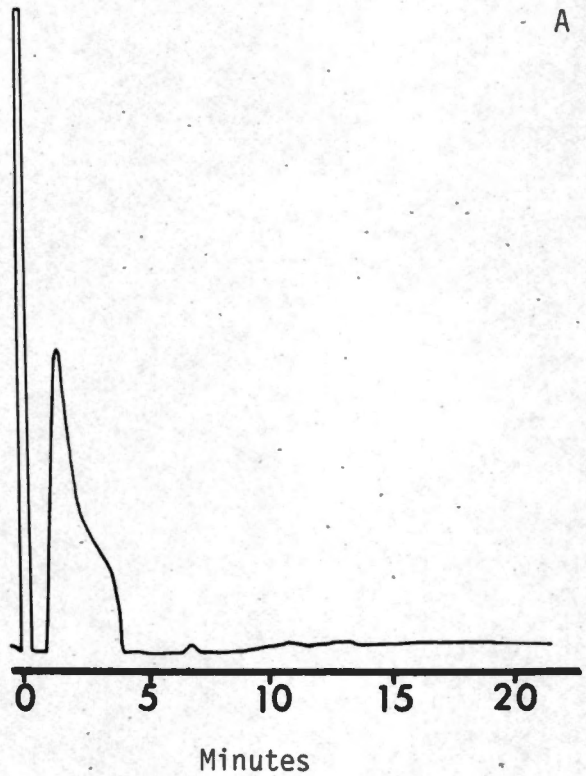
[e]Significant at $P<.05$ level.

MASS	ABUND	MASS	ABUND
39	2.1	105	4.6
41	19.6	111	4.0
41	1.8	112	1.6
43	24,3	115	4.0
53	1.2	119	2.6
55	19.9	128	2.9
56	6.5	129	2.5
57	45.0		
		133	2.6
67	6.7	141	5.4
69	12.3	145	7.8
70	6.3		
71	21.6	155	1.6
		156	2.7
77	2.1		
79	1.9	161	2.5
81	8.4		
82	2.2	177	8.4
83	8.8		
85	13.2	189	2.0
91	6.2	205	100.0
95	5.4	206	15.8
96	2.3		
98	2.8	220	26.4
99	2.6	221	3.6
		>PAUSE	

Figure A-1. GC-Mass spectrum of composited cabbage sample compound peak number 5 identified as BHT.

MASS	ABUND	MASS	ABUND	MASS	ABUND
39	.5	115	3.5	221	4.3
41	2.3	117	1.5	222	.2
43	.4			>PAUSE	
		119	2.8		
51	.1	121	2.5		
53	.5				
55	1.3	137	.3		
57	5.9	143	1.0		
58	.2	145	9.2		
65	.7	147	1.2		
67	1.0	149	1.7		
69	.4	159	1.0		
77	1.9	161	2.9		
78	.4	163	1.3		
79	1.2				
81	2.0	177	9.9		
		178	1.1		
91	4.1				
92	.3	189	3.1		
93	.4	190	.3		
95	1.8				
		203	.4		
105	4.7	205	100.0		
106	.4				
107	.5	220	26.4		

Figure A-2. GC-Mass spectrum of BHT.



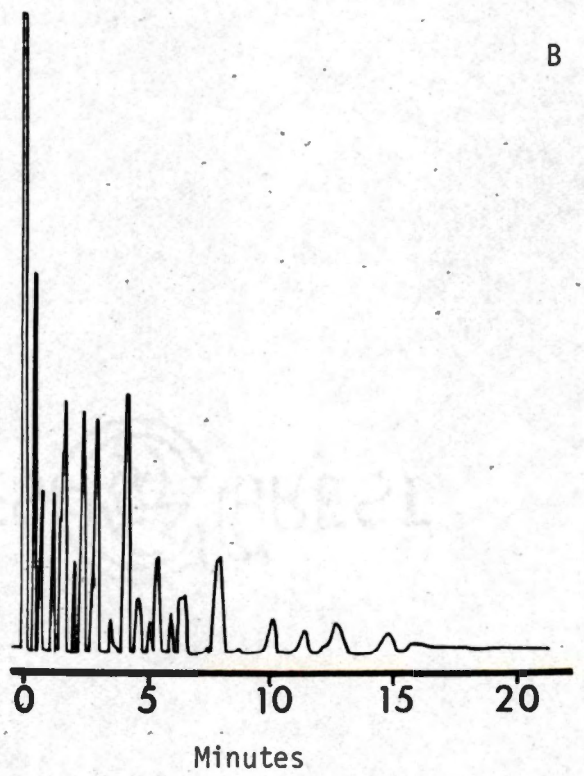


Figure A-3. Representative chromatograms of interference with pesticide analysis from cabbage sample (A) without alkaline hydrolysis and (B) with alkaline hydrolysis. (Oven temp. 205°C)

VITA

Adriance Lorene Prater was born on July 2, 1953, in Knoxville, Tennessee, to William Hugh and Lorene Loy Prater. Upon graduating from Farragut High School, Concord, Tennessee, in June of 1971, she entered the University of Tennessee, Knoxville, and in June 1975 received a Bachelor of Science degree in Home Economics with a major in Food Science. From February 1976 to December 1977, the author worked in the Department of Food Technology and Science at the University of Tennessee, Knoxville, as a laboratory assistant. She enrolled in the graduate school at the University of Tennessee, Knoxville, in January 1978 and began work toward a Master of Science degree in Food Technology and Science.

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